Vol. 31, No. 1 March, 1944

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF APPLIED BIOLOGISTS

BY

W. B. BRIERLEY

AND

C. T. GIMINGHAM

PUBLICATIONS COMMITTEE

F. C. BAWDEN F. R. PETHERBRIDGE

R. W. MARSH I. THOMAS

W. C. MOORE

CAMBRIDGE UNIVERSITY PRESS

LONDON: BENTLEY HOUSE

CHICAGO: The University of Chicago Press
(Agents for the United States)

BOMBAY, CALCUTTA, MADRAS: Macmillan

All rights reserved

PRINTED IN GREAT BRITAIN

The Association of Applied Biologists

President
PROF. W. BROWN, M.A., D.Sc., F.R.S.

Vice-Presidents
E. W. MASON, M.A. H. W. MILES, D.Sc.

Hon. Treasurer
W. C. MOORE, M.A.
Plant Pathological Laboratory,
Milton Road, Harpenden, Herts.

Hon. Editor (General and Botanical)
PROF. W. B. BRIERLEY, D.Sc.
University of Reading,
Berks.

Hon. Editor (Zoological)
C. T. GIMINGHAM, B.Sc., F.I.C.
Plant Pathological Laboratory,
Milton Road, Harpenden, Herts.

Hon. Secretary (General and Botanical)
W. P. K. FINDLAY, D.Sc.
Forest Products Research Laboratory,
Princes Risborough, Bucks.

Hon. Secretary (Zoological)
H. F. BARNES, M.A., PH.D.
Rothamsted Experimental Station,
Harpenden, Herts.

Council

L. A. ALLEN, PH.D.
G. M. BATES, D.Sc.
F. C. BAWDEN, M.A.
J. R. BUSVINE, PH.D.
G. V. B. HERFORD, B.A.
E. W. MASON, M.A.
H. W. MILES, D.Sc.
H. SHAW, PH.D.
W. STEER, B.A.
I. THOMAS, PH.D.
W. A. R. DILLON WESTON, M.A., PH.D.
MISS A. P. WILSON, A.R.C.S.

CONTENTS OF VOL. 31, No. 1

PAGE

1. Pollution of the rivers of West Wales by lead and zinc mine effluent. By LILY NEWTON. (With 1 Text-figure) 1
2. Physiological cotton wilt in the Sudan Gezira. By A. S. BOUGHEY, B.Sc., PH.D. (With Plate 1 and 5 Text-figures) 12
3. The nutrition of the pea. By R. M. WOODMAN 19
4. The nutrition of vegetables in sand. By R. M. WOODMAN. (With 21 Text-figures) . . 22
5. Notes on basal rot of narcissus. III. Eradication of the disease from narcissus stocks by repeated use of formalin in the hot-water bath. By LILIAN E. HAWKER. (With Plate 2) . 31
6. The relationships of some viruses causing necrotic diseases of the potato. By F. C. BAWDEN and F. M. L. SHEFFIELD. (With Plate 3) 33
7. The 'phloem necrosis' virus disease of tea in Ceylon. I. Introductory account, symptoms, and transmission by grafting. By T. E. T. BOND. (With Plates 4 and 5) 40
8. An unusual correlation between insect damage and crop harvested. By C. H. GADD, D.Sc. (BIRM.) 47
9. Studies of wireworm populations. I. A census of wireworms in pasture. By GEORGE SALT, SC.D., and F. S. J. HOLLICK, PH.D. (With 7 Text-figures) 52
10. The use of toxic polynitro derivatives in pest control. II. The estimation of dinitro-*o*-cresol in winter washes. By R. F. BATT, H. MARTIN and R. L. WAIN 64
11. The application of the probit method to toxicity test data adjusted for mortality in the controls. By D. J. FINNEY, M.A. 68
12. Proceedings of the Association of Applied Biologists: 1 October 1943. Symposium on control of insects attacking stored foodstuffs:

    The organization and work of the Pest Infestation Laboratory of the Department of Scientific and Industrial Research. By G. V. B. HERFORD 75

    Factors influencing the course of an insect infestation in bulk wheat. By T. A. OXLEY and R. W. HOWE. (With 2 Text-figures) 76

    Behaviour of tyroglyphid mite populations in stored grain and flour. By M. E. SOLOMON 81

    The role of the chemist in research on fumigation. By W. BURNS BROWN . . . 81

    Control of the granary weevil with finely ground mineral dusts. By E. A. PARKIN . 84

Pollution of the rivers of West Wales by lead and zinc mine effluent

BY LILY NEWTON, *University College of Wales, Aberystwyth*

(With 1 Text-figure)

The chemical composition of river water depends in part upon the geology of the hinterland. Heavy rain washes away the surface soil or otherwise changes the composition of the water by increasing the volume of soluble salts which it contains. The fauna and flora of a river vary seasonally; in addition, both individuals and species are affected numerically by the chemical composition of the water. These phenomena have had serious consequences in Wales, where the main watershed of the drainage system is situated in an area where lead and zinc have been mined intermittently from prehistoric times until to-day (Jones, 1922). The salts of both these metals are carried into the streams and their effects have been investigated during the past 20 years by a number of workers in the University of Wales, mainly in the Departments of Chemistry, Zoology and Botany of the University College of Wales, Aberystwyth.*

Lead and zinc were mined in Cardiganshire in the time of Queen Elizabeth after the foundation in 1568 of the 'Society for the Mines Royal'. In and after 1690, new mines were opened and further workings took place. By 1747 there were records of deleterious effects of the mines upon live-stock. A long period of decline followed, and between 1872 and 1922 numerous mines closed down, though there was a recrudescence during the 1914–18 war period.

The degree of pollution due to the mines varied with the mode of treatment of the ore. The introduction of the Cornish ore-crushing method led to the formation of slime pits very rich in lead and zinc; water passing from these pits was especially deleterious to the fauna and flora of the rivers.

In 1874 the Rivers Pollution Commissioners (Anon. 1874) stated that land and live-stock suffered injury from lead pollution in the valleys of the Ystwyth, Rheidol, Clarach and Dyfi. At that time the serious effects of mine effluent on the rivers were recognized, but there was little precise knowledge of the underlying causes and no remedial measures were known. With the closing of the majority of the mines some of the rivers began a long, slow recovery, which had periodic setbacks through the spasmodic opening of individual mines. Other streams remained polluted and the whole area, comprising the headwaters of the Dovey, Clarach, Rheidol, Ystwyth, Teify and Towy, was more or less affected.

The rivers concerned (Fig. 1) have a westerly or south-westerly course and reach the sea in Cardigan Bay, with the exception of the Towy, which flows into Carmarthen Bay after following an almost southerly course. Their headwaters are situated in Mid-Wales in regions where lead (galena) and zinc (blende) have been mined since early times.

Griffith & Angus (1917) and Griffith (1919) indicated many ways in which mine refuse could be carried, and pointed out that the Ystwyth, Rheidol and Clarach when in flood deposit enormous quantities of mine detritus upon the land in the lower river valleys, where the conditions favour sedimentation. Griffith (1919) suggested that the pollution of the river water was not only due to the presence of effluent but to the washing into the rivers of sand and slime from mine refuse heaps.

In 1923 the investigation of the Teify watershed was considered (Anon. 1923). The river had then improved, and no lead mines were in active operation, but there was a possibility of new mines being opened near the headwaters. If this proposition materialized, the river would afford an interesting study of consequent biological changes, and it was suggested that the effect of rain drainage from old mine dumps entering the river should be investigated biologically as well as chemically.

Carpenter (1924) studied the pollution of streams in the Aberystwyth district consequent on lead-mining, since the paucity of fish life in the river was thought to be caused by the clogging and inflammation of the gills, due to adhesion of particles of galena grit. The animal species of polluted and unpolluted streams were compared. It was established that the lead-polluted streams exhibited a remarkably poor fauna, and that the determining factor in causing pollution was not galena grit but the presence of lead salts in solution, the latter term covering true molecular solution and also colloidal condition. The lower Rheidol and Ystwyth had markedly improved since 1922, both in regard to plant and animal species. Furthermore, experiments with minnows in the lower Rheidol proved that though fish could live in the river in a dry season,

* A complete set of records was destroyed by enemy action and the present paper has been compiled after the data had been collected from scattered sources and placed in the Library of the University College of Wales, Aberystwyth.

Fig. 1. Map showing the relative positions of the lead mines of Cardiganshire and the main watershed.

they died in flood-time when dissolved lead salts were present.

Carpenter (1925) demonstrated that a high responsibility for pollution lay with the dissolved metallic content of mine effluent. The biological evidence showed that the safety of stream life was incompatible with the free entry into rivers of mine waters bearing lead in solution. The treatment of the lead washings by slime pits and the screening of dumps from weathering were ineffective as long as the water entering the river contained dissolved lead.

At that time, the Ystwyth had suffered fresh pollution owing to zinc-dressing work, but the Rheidol continued to improve. Carpenter (1925) demonstrated experimentally that when fish died in the river, a thick mucus formed around their bodies and especially over the gill filaments. She concluded that the reduction of pollution in drought was due to the fact that the river-ward slopes of the dumps were washed clean of soluble lead, though weathering presumably exposed fresh supplies of material periodically, which were carried into the river in flood-time. It was suggested that the establishment of settling pits, as required by the Ministry of Agriculture and Fisheries, did not meet the situation, since the pollution did not depend on the grit, the nature of the lead salt, or the strength of the molecular solution, but on the total quantity of lead present, including that in the colloidal condition. There was a discrepancy in the time factor of the results, natural flood-waters acting more quickly than the same strength solution made in the laboratory; the presence of zinc was suspected, and was confirmed in January 1925.

Coates & Hinkel (1925) confirmed the polluted condition of the Ystwyth and considered the position of the Teify in regard to the possible reopening of the Esgair Mwyn mine near its headwaters. The effectiveness of the settling-pond system which Carpenter had questioned was investigated in the field and laboratory, and it was concluded that lead, and to a much greater extent zinc, were readily taken up into solution by water, under the conditions obtaining in the ore-dressing process and on the weathering of the finely divided galena. The ore-dressing effluent might be expected to contain dissolved lead to the extent of about 3 p.p.m. (and much more zinc), and no system of settling pits removed the dissolved lead.

Coates & Hinkel also stated that a saturated solution of lead sulphate contained nearly 3 p.p.m. lead, and that this was approximately the same as the lead in solution when the galena from the mines, which also contained zinc, was treated with water. This was inaccurate, as James (1927) demonstrated that a saturated solution of lead sulphate contained 30 p.p.m. The presence of excess sulphate depressed the solubility (see Purdum & Rutherford, 1933), so that the solution obtained on the oxidation of galena on the mine dumps contained about 3 p.p.m., owing to the presence of as much as 200 p.p.m. of sulphate in the detritus.

Zinc oxidizes to sulphate more readily than lead, and zinc blende oxidizes in moist air more readily than galena. At all times zinc was present in the polluted streams in higher proportion than lead. Coates & Hinkel (1925) confirmed that cellulose in its various forms removes lead from solutions, while commercial permutit removes both lead and zinc.

Coates & Hinkel stated that in practice the suspended matter from the mine effluent should be removed by sedimentation in settling ponds, that the fine suspension should be removed by means of a filter bed, and that earth or peat moss might be expected to remove the dissolved lead but would be less effective for zinc. A permutit filter would remove the last traces both of lead and zinc. They emphasized that rain water flowing over the weathering dumps should not pass directly into the river.

In the summer of 1924, mining activities recommenced at the Esgair Mwyn mine near the headwaters of the Teify, and the Marchnant brook, which had completely recovered from pollution, became seriously affected. Carpenter (1926*a*) found that by the summer of 1925 the mollusc *Ancylus fluviatilis* (*Ancylastrum fluviatile*) and trichopteran larvae had disappeared, which accorded with Ortmann's results in Pennsylvania (1909), where the disappearance of molluscs was regarded as the first index of the pollution of a stream by lead-mining and the disappearance of trichopteran larvae marked the second phase.

In 1926, the relevant local Committee considered the condition of the rivers, and it was recognized that the Ystwyth had not recovered from pollution despite a long period of inactivity in the lead-mines. The reopening of the Gwaith Goch works, in 1924, caused a further setback. The lower reaches of the Rheidol, at one time barren, began to recover, until the reopening of the Erwtomau mine caused serious pollution. Furthermore, the Marchnant and upper reaches of the Teify, which had completely recovered between the years 1918 and 1924, both gave cause for apprehension with the reopening of the Esgair Mwyn mine. The general position at that time was that under the Salmon and Freshwater Fisheries Act of 1923 it was illegal for anyone to cause a condition endangering fisheries in a river. Owners of mines were liable to prosecution if the effluents contained traces of soluble metal which endangered fish life, but since practical chemical means of dealing with the effluent were unknown, it was scarcely fair to penalize the owners for pollution which could only be prevented by closing the mines.

Carpenter (1926*a*) studied the rivers during the

process of their slow recovery and discovered the following sequence:

(i) Establishment of a limited cryptogamic flora (including *Lemanea*, *Batrachospermum* and a few bryophytes) and of a very sparse invertebrate fauna consisting almost entirely of insect larvae. Oligochaetae, Mollusca, Platyhelmia and (among insects) Trichoptera still unrepresented.

(ii) Establishment of chlorophyceous algae and invasion by oligochaetes, turbellarians and caddis larvae, as well as additional types of insect groups already represented, with fishes as occasional visitors from neighbouring waters.

(iii) Growth of flowering plants such as *Callitriche verna* and *Ranunculus aquatilis*, coupled with a large increase in invertebrate types, Mollusca being added, including *Ancylus fluviatilis*, and finally fishes becoming established.

These phases could be traced successively in a single reach of the river as time elapsed after closure of the mines, and they could be followed through successive reaches of the same stream, passing progressively from the source of pollution. Phase (i) was present when the river water held in solution small but appreciable quantities of lead up to 0·4 p.p.m.; phase (ii) was associated with an intermittent chemical pollution, the supply of soluble lead being renewed at flood-times; and phase (iii) supervened when intermittent renewal ceased, in some cases only after a lapse of several years. The phases of deterioration repeated the recovery phases in reverse order. It was thought that the lethal action of the lead was probably accelerated by the presence of traces of zinc.

The Marchnant brook after 1 year's partial activity at the Esgair Mwyn mine showed definite signs of pollution. It was demonstrated experimentally that the solution of heavy metals by the direct action of water upon solid mine waste was the source of pollution, while the solid matter was not considered deleterious to the fauna of the streams. Work by Reese (1937) and Jones (1940*b*) modified this conclusion.

In 1926 the position was further complicated by technical advances in the flotation method of separating ores which involved the discharge of oil and mineral acid into the rivers, thereby increasing the sources of pollution.

It was recognized that the possible answer to the whole problem lay in treating the effluent and preventing pollution of rivers, in a manner which would be a reasonable financial proposition for the mine-owners. Carpenter (1926*b*) established a number of new facts concerning the lethal action of lead salts on fishes. She demonstrated experimentally:

(1) The reaction to small quantities of lead salts in solution was constant in type for trout, minnows and sticklebacks, but was most rapid with the two latter.

(2) The speed of the reaction varied, approximately, inversely as the size of the fish.

(3) There was some evidence that sexually mature fishes succumbed more easily than immature fishes.

(4) The cause of death was the formation of a colloidal precipitate of the heavy metal upon the gills of the fishes, which impeded the respiratory function of the latter and ultimately caused death by suffocation.

(5) If the solution was so dilute that the coating of the gills was incomplete, and if subsequently the water was free of the heavy metals, the fish were able to throw off the mucus and recover.

(6) The toxic action of salts of zinc, cadmium, iron and copper was essentially similar to that of lead *at high concentrations*: low concentrations were not studied.

It was observed that other workers had found a slow growth rate in fish which had lived in water containing heavy metals, too dilute in concentration to be lethal. Chemical analysis of the body of the fish, after removal of the lead from the gill plates, failed to reveal the presence of lead.

James & Jones (1928) reported on the Esgair Mwyn mine, a source of pollution of the upper waters of the Teify. A series of small settling pits existed, about 50 yd. in total extent. After leaving these pits, the water was joined by a larger volume of unpolluted water from the power-house and flowed into the Marchnant brook, a tributary of the Teify, about a mile from the settling ponds. Just below this confluence, water was taken from the Marchnant and carried by a long leat to the Llwyn Llwyd mine, on the other side of the valley. When work was in progress, the effluent from the Llwyn Llwyd mine discharged into the Ystwyth. By this system both the Teify and the Ystwyth received pollution, and it was recommended that all the polluted water from the Esgair Mwyn mine should be conducted by a small aqueduct to the leat and the Llwyn Llwyd mine and thence to the already polluted Ystwyth, thus keeping the Teify free from pollution.

The system of settling pits did not reduce the effluent, and the water in the leat flowing into the Marchnant brook contained 3 p.p.m. lead as well as zinc in solution. James & Jones (1927) ascertained that neither the addition of sodium silicate to the dressing water nor a change in the *p*H of the water prevented lead from passing into solution.

They found that the filtration of the mine effluent through a 6 in. peat-bed or filter removed all suspended material and dissolved the lead, though the bed needed renewal from time to time. It remained efficient until the peat had adsorbed 2·9% g. by weight of lead. If, previous to filtration, the effluent was treated with enough sodium silicate to raise the *p*H to 7·2, the main proportion of the zinc present was also removed, together with any traces of copper and iron. A solution of zinc passing through a

sufficient thickness of peat would probably lose the metal, though experimentally it had been demonstrated that zinc passes through thin layers of cellulose without being adsorbed. Galena, agitated for long periods with air and water, became oxidized to lead sulphate. It was clear that the weathering of the surface of old dumps and slime pits gave rise to the presence of considerable amounts of lead sulphate which were washed into the streams, particularly in flood-times.

James & Jones (1928) carried out a large-scale experiment. A filter bed and adsorption bed were set up at Esgair Mwyn mine where the water left the final settling pit, and though there were experimental difficulties, especially in the filter bed, it was proved that a peat adsorption bed is an efficient means of removing lead, and it was estimated that the bed would retain its efficiency for a period of 12 weeks. Later, peats from Northumberland and Ireland were found to be equally efficient. They proposed to treat the Esgair Mwyn effluent by a series of filter and peat adsorption beds and estimated that the whole of the effluent could be treated by beds with ten times the capacity of those used experimentally. A filter bed 6 ft. sq. could deal with 600 gal./hr. The purified effluent could then be discharged into the lower Marchnant through the Nant-y-Garw.

The appendix to the Report pointed out the serious pollution of the Nant-y-Garw before it joined the Marchnant, and also drew attention to the pollution of the Nant-y-Gwyddil.

The complete treatment of the effluent was never achieved because a hard frost damaged the power plant and the mine was flooded. Subsequently, the price of lead fell and the mine closed down.

The water which continued to flow into the Marchnant constituted a source of pollution. That the Teify was not seriously affected in its lower reaches is almost certainly due to the fact that shortly after leaving the polluted area it flows through the site of an old glacial lake, now occupied by a peat bog. The peat forms a natural adsorption bed, and the water of the river is completely free from metallic pollution when, after meandering through Tregaron Bog, it makes its way through the remains of the old moraine at Tregaron.

Concurrently with the work of James & Jones (1927, 1928) researches were made by Roberts & Leach (1929) in the Pennine area. They pointed out the damaging effects of mine effluent both chemically and mechanically by covering the bed of streams with silt and slimes which inhibit the growth of aquatic vegetation and insects and clog and render useless the potential spawning beds of salmon and trout. They considered that filter beds of the type which James & Jones had used experimentally clogged readily, and because of their irregular interstices and the slow rate of percolation of water through them, were impracticable on a larger scale. They considered that filters consisting of parallel plates were the most efficient but were, in practice, too costly. They stated that the best remedial measures were the application of sedimentation to remove the suspended material followed by the utilization of a peat filter as designed by James & Jones to remove the colloids.

In connexion with sedimentation it was pointed out that precipitants could be used, but that their addition needed great care as to the quantity required to be controlled and that the sediment must be removed, otherwise the material might go into suspension again. A small quantity of lime would neutralize the charge on the particles and cause them to settle. They recommended the addition of milk of lime and a concentrated solution of ferrous sulphate in a quantity that would have to be determined for each mine. In this way the solid matter could be removed with the use of properly designed settling pits.

In 1929 and the following years periodic field surveys of the Cardiganshire rivers were carried out under the direction of T. C. James, R. D. Laurie and L. Newton, with the assistance of members of their staffs and other research workers, to ascertain the degree of pollution which continued to occur and the faunistic and floristic conditions of the streams. The following is a summary of the results.

TOWY. *Nant-y-mwyn mine* (James & Hudleston, 1931). At this mine, which is on the upper reaches of the river, a flotation process was introduced. The mine dealt with about 100 tons of ore daily, which was ground to a very fine powder and passed through a separation plant. The slimes then passed into settling pits, but these were not well built and, in 1930, the wall of one of the main pits burst, and the Nant-y-mwyn valley down to its confluence with the Towy was contaminated with slimes. The water emerging from the settling-pits system contained 3 p.p.m. lead, and the Towy itself, 100 yd. below the entry of the tributary, 0·025 p.p.m. dissolved lead. The seriousness of the situation was clear as Carpenter (1926*b*) had shown 0·3 p.p.m. to be lethal to certain fish. The Nant-y-mwyn mine closed and the management attempted to cover the old slime pits with earth, with a view to their subsequent colonization. This was not completed and the mine would be a source of pollution to the Towy if there was any break in the retaining structures.

DOVEY (Laurie & James, 1931; Jones, 1941). In the higher reaches of the Twymyn, a tributary of the Dovey from the Dylife and Pont-ddol gadfan mines, the river was heavily polluted by water percolating through old dumps, but the volume of polluted water was not large enough to affect the Dovey. Trout occurred from Llanbrynmair to the sea and salmon were plentiful in the lower reaches.

TEIFY (James & Laurie, 1931). Examination of the headwaters of the river showed no serious pollution except that a small stream issuing from the

dumps just below Strata Florida Abbey was heavily polluted (45 p.p.m.) by zinc. The absence of *Ancylus fluviatilis* below this mine, and in the Marchnant and the Meurig, suggested intermittent pollution of these streams. The pollution diminishes and the passage of the river through Tregaron Bog removes all traces of metallic pollution.

CLARACH. The Nant Silo, one of the head streams of this river, flows through a lead-mining area and is polluted by effluent. It yielded only twenty-two animal species, mostly insect larvae, when investigated by Arthur (1937–8, unpublished). The Capel Dewi tributary, which flows through an unpolluted valley, contained forty-six species, including trout, sticklebacks, eels, tadpoles, leeches, molluscs and the fresh-water shrimp, *Gammarus*, which is sensitive to pollution. The addition of clean tributary streams augments the river in its lower reaches where a normal fauna exists, including trout.

YSTWYTH. This remained a seriously polluted river. In 1936 it was proposed to start ore extraction from the old dumps at Cwm Ystwyth, and the attention of the Cardiganshire County Council was drawn to the necessity for ensuring that efficient precautions were taken to prevent still further pollution of the river. Jones (1940*a*) surveyed the River Ystwyth, which he found polluted by lead from a trace up to 0·5 p.p.m. and by zinc from 7 to 12 p.p.m. when at normal level. The river bed was silted with quantities of mine detritus and the main stream was completely devoid of phanerogams, while bryophytes were present only in small amount. The fauna was limited to sixty species, fifty-three of which were insects, highly resistant to metallic pollution. The remainder were water-mites and flatworms. The absence of fish can be attributed to the presence of zinc pollution. Sewin were reported to ascend the river as far as Llanfarian bridge, where they were sometimes found dead or dying. Brown trout occurred plentifully above the Cwm Ystwyth mine which is the first source of pollution. Jones drew attention to the long period of pollution even after the closing of the mines, and suggested the possibility of diverting the Frongoch stream so that it did not flow through the mines. Since diversion of polluted waters *into* the Ystwyth had previously been the policy adopted, no action was taken. In 1941, war conditions led to an investigation of old mines and mine levels with a view to the development of the area as a source of zinc ores, but no mining ensued. A subsequent survey by James & Newton (Anon. 1942) of the leats and mines in the area concerned led to the opinion that it was impracticable to divert the Frongoch stream in any way that would prevent the water from ultimately reaching the Ystwyth.

RHEIDOL (James *et al.* 1932). Much improvement was observed in the condition of the river since 1921 (Carpenter, 1924).

The Rheidol mines, the Melindwr mine and the mine at Erwtomau ceased work between 1922 and 1927, and there was a gradual improvement in the flora and fauna of the streams existing in the lower reaches. The backwaters teemed with sticklebacks and tadpoles, occasional salmon and sewin were recorded near Parc-y-llyn, and trout were taken between Aberffrwd and Aberystwyth. The water was slightly polluted by lead and zinc, though the amount was not considered sufficient to constitute a menace to fish life, except between Cwm Rheidol and Rheidol falls on the main river. The Melindwr tributary was still polluted by the mine through which it passed.

The survey showed that the Rheidol on the whole was passing through recovery phases, though a few areas remained which were causing pollution. During the ensuing 10 years the condition of the river was investigated with a view to suggesting remedial measures. The work is reviewed under subject headings.

A. CHEMICAL INVESTIGATIONS

The river was tested at intervals along its whole course, and it was concluded that the water was unpolluted from the source to the mouth of the gorge just above the Cwm Rheidol mine. The tributaries Castell and Tuen were also almost free from pollution. The Cwm Rheidol dump, however, constituted a source of pollution. The 'red' stream issuing from the dump and discharging into the river contained 30 p.p.m. zinc, and though the lead content was low, it contained free sulphuric acid in a concentration of 100 p.p.m. Its high iron content was largely deposited as hydrated iron oxide. There were no slime pits used for settling the ore, and the river was poisoned practically to the Rheidol falls. The small streams from Cwm Brwyno and the Erwtomau mine were also polluted. Though the Melindwr passed through the Goginan mine, the water was little affected chemically.

It was suggested that the 'red' stream might be made to pass over limestone to destroy the acid, and then through a peat bed to remove the lead: this treatment would also partially remove the zinc. Treatment of the water by passing it over limestone chippings was also recommended, as had proved effective for the Aberystwyth water supply. The mines were not working, and the treatment would probably have been effective.

In July 1938, the mines were reopened and sudden large outrushes of water during the preliminary operations discharged a large quantity of heavily polluted water into the Rheidol. The bed of the 'red' stream was washed into the river. The management subsequently erected a series of settling pits from which the effluent ultimately seeped into the river. The effluent held lead and zinc in solution,

but the large porous heap of detritus through which it seeped held the water, so that even after heavy rain the volume which reached the river at any one time was never very considerable. The mine closed shortly afterwards, and the river is gradually recovering even in the region between the mine and Rheidol falls.

B. BOTANICAL INVESTIGATIONS (Newton *et al.* 1936)

The work had a threefold purpose: to ascertain the effects of pollution on the attached and free vegetation of the streams; to follow up a suggestion that some of the dumps could be sealed by vegetation; and to ascertain whether the unsightly areas could be clothed by vegetation. In addition, since cellulose adsorbs lead very easily (Coates & Hinkel, 1925), as had been demonstrated by peat-filter experiments, it would be a partial solution of the problem in certain areas if a band of deciduous vegetation could be established on the stream margin of some of the dumps, so that surface drainage water would need to percolate through humus before reaching the stream. Much depended on the toxicity of lead and zinc to plants, since the leaching out of these metals was the cause of pollution. Griffith (1919) pointed out the serious effect of lead on plants and animals, and it was clear that before remedial measures could be taken the actual effect of lead and zinc on plants under controlled conditions must be ascertained, and also the effect of lead and zinc pollution on the microflora of the stream.

Reese (1937) worked on the latter problem. Glass slides were anchored in the Rheidol and its tributary the Melindwr, above and below sources of pollution, and periodic counts were made of the microflora. The results were checked from time to time by examining the flora on pebbles, and so ensuring that the data gave a correct estimation of the condition of the river. A well-defined periodicity occurred during the winter months, but although there was less development below the sources of pollution, the streams were not devoid of organisms. The silt which washed into the river in flood-time, immediately below the Goginan mine, was considered to be a causative factor in producing the barren appearance of that stream below the mine, and similarly the silt brought down by flood-water caused a scouring of the river bed, and destroyed, at least temporarily, the diatoms. The more firmly attached species, particularly encrusting forms attached by a large part of their surface, were unaffected. The badly polluted 'red' stream, which entered the river at Cwm Rheidol, appeared to have a deleterious effect on the microflora at that point. These data on the condition of the microflora in the river were of value, though it was not possible to discriminate between the effects of lead and zinc.

A study of the macrophytes of the river showed the paucity of flowering plants in, or near, the stream immediately below the sources of pollution, while the bareness of the dumps formed a striking feature of the valley at all times. Surveys and field and laboratory experiments were made to ascertain the effects of lead and zinc on flowering plants, and to determine the distribution of vegetation in and near the river and its tributaries, and also on the old dumps. The river water contained only 0·03–3 p.p.m. lead, but the zinc reached 36 p.p.m. in the stream flowing through the Cwm Rheidol mine and entering the river at that point, so that all experiments were conducted in triplicate, using lead, zinc, and lead and zinc together.

Surveys of the plants growing on the old workings and dumps at Goginan made by E. A. Lewis & Herbert Jones (unpublished) demonstrated the paucity of species and individuals. *Thlaspi alpestre* was absent, though it occurred in the Ystwyth valley in polluted areas and was a conspicuous feature of the colonized old workings of the Mendip Hills.

Hooper & Newton (1935) and Hooper (1937) showed that lead had only a very slight toxic effect on dwarf French beans grown in single salt cultures with lead sulphate, or in the presence of nutrient salts and lead sulphate. This was true even when a saturated solution of lead sulphate was used, containing 30 p.p.m. lead. Spraying experiments indicated that the presence of lead on *Ulex*, *Lupinus* or Carter's '50 days' dwarf French bean plants had no deleterious effects. It was concluded that the presence of lead was not the direct cause of the barren nature of the mine dumps.

Newton *et al.* (1937) and Davies (1941) showed that zinc sulphate is toxic to plant growth, and that its presence on the mine dumps is one reason for the slight vegetation and the pollution of the rivers. The results suggested that phosphate deficiency might be a limiting factor, since any phosphate in the soil would combine readily with the zinc present and be unavailable for plant growth: liberal phosphate dressings on the mine dumps might therefore have a beneficial effect on the establishment of vegetation.

Newton & Glencross (1940) carried out two series of experiments: (*a*) water cultures to ascertain the combined effect of lead and zinc on dwarf beans; and (*b*) pot cultures to determine the effect of lead on beans and clover, grown in the presence of excess lime and phosphate. Series (*a*) gave results similar to those obtained for zinc alone, and further confirmed former observations of the liability of plants grown in the presence of zinc to fungal attack, and of the possibility of phosphate deficiency being one cause of the paucity of vegetation. In series (*b*) beans and clover were used as examples of plants comparatively resistant and sensitive respectively to the presence of polluting metals. The presence of lead

affected the germination of the seeds in both cases, but the experiments proved that the addition of excess calcium carbonate, and also of excess calcium carbonate and basic slag, materially reduced any deleterious effect of the lead on the subsequent growth of the plants.

Newton *et al.* (1938) continued this work, using zinc and also lead with zinc, but the results are not yet available. The preliminary results indicated that dressings of superphosphate and potassium sulphate were beneficial when added to the soil of the mine dumps, but that ammonium sulphate was markedly deleterious.

A survey by Jones & Lloyd (unpublished) of the flowering plants of the Rheidol and Melindwr in 1938–9 showed that in the lower reaches of the Rheidol vegetation was luxurious. They established that *Ranunculus aquatilis* is very sensitive to pollution, and that the plant ceases to occur where tributary streams bring polluted water into the main river.

The aggregate results of the experiments showed that, botanically, zinc is a more serious source of pollution than lead, and that phosphate deficiency is probably one of the causes of the poor development of vegetation of the dumps.

The presence of up to 1 p.p.m. of zinc was shown to stimulate the growth of dwarf French beans, but in the presence of 1–10 p.p.m. the toxicity increases sharply with the concentration, the plants being stunted and liable to fungal attack, a fact noted in other plants growing on the dumps. There is also a definite indication of the value of the addition of lime and phosphate to the soil in attempting to grow plants in areas influenced by mine effluent.

Field experiments were conducted at Goginan, where it was established that one of the most serious causes of the small amount of vegetation is the blown material from the old slime pits, which is carried into the air in clouds by easterly winds and deposited on the adjacent land. This is a serious and continuous menace, irrespective of whether mining is in progress. The leaching of lead and zinc from the surface material of old slime pits is a phenomenon which reduces the incidence of pollution so long as the mines are not working and the dumps are undisturbed, but time does not lessen the damage caused by blown material. Experiments conducted at Goginan showed that this material enveloped the vegetation in the vicinity, and in the case of young pine trees it kept pace with the annual growth so that only the uppermost whorl of branches was ever exposed. The treatment of the large surface of fine material which occupies the position of the old slime pits, by covering it with soil or spraying with some binding substance such as bitumen, would remove this source of pollution. If the soil method were adopted colonization by vegetation would be possible, and the removal, at all events in part, of the unsightly character of the old mines would be effected.

C. Zoological investigation (Laurie & Jones, 1938)

Though the Rheidol improved greatly, there was slight pollution in flood-time, even in the main river. During a comparatively dry period, oxidation of galena and zincblende took place on the dumps, and the resulting sulphates could be seen as a white powdery substance on the surface. In a period of flood, these more soluble sulphates were washed into the river, along with additional detritus, thus causing intermittent periods of metallic pollution. Jones (in James *et al.* 1932) ascertained the relative resistance of stream life to intermittent and continual pollution: he demonstrated that it might be expected that snails and fish would disappear first, then tadpoles, newts, etc., then mayfly and dragonfly larvae and leeches, and lastly, insects and flatworms.

R. D. Laurie (in James *et al.* 1932) demonstrated the great increase in the number of species and individuals in the river between the years 1919 and 1933. Jones (1936) showed that the stickleback, which is very sensitive to lead, was killed after 14 days' exposure to 0·1 p.p.m. lead. The Rheidol generally contains 0·02 p.p.m. lead and has only received 0·1 p.p.m. for some years even in flood-time. It was apparent that as far as lead was concerned, the chemical condition of the river offered no barrier to the presence of either a rich invertebrate fauna or of some fish, though the concentration toxic to trout is unknown. Trout were caught in the river when the pollution was 0·1 p.p.m., and although the fish appeared healthy, there was enough mucus attached to the gills to give a lead reaction.

Further work by Jones (1936) demonstrated that periodic pollution of a river might occur without seriously harming its fauna, provided that the period of the pollution was not more than a few days. His results suggested that from July 1931 to May 1936 the lead content of the Rheidol was not normally greater than 0·05 p.p.m., though it may have risen to 0·1 p.p.m. for short intermittent periods.

Jones (1937) further studied the effects of zinc on fish. The results were almost exactly the same as for lead, down to a concentration of 1 p.p.m. At the lower concentrations the toxicity fell more rapidly, and became indefinite at 0·3 p.p.m. Experiments using a mixture of the two metals in the proportion of 1 zinc : 5 lead, as was approximately present in the river, showed results very similar to those obtained when using zinc alone.

Jones (1937) summarized his results and demonstrated that for the stickleback the critical concentrations of lead, zinc and copper were as follows: lead, 0·1; zinc, 0·3; and copper, 0·01 p.p.m. At these concentrations, the stickleback could survive, but higher concentrations were lethal. Copper was investigated in connexion with the pollution in the valley of the Mawddach, 2½ miles from Dolgelley.

Jones (1938) examined the effect of adding calcium nitrate to polluted water, and found that if it was added so that the calcium content was 50 mg./l., the effects of the lead and zinc present were annulled. The effect appears to be that the presence of calcium prevents the reaction between the mucus secreted by the gills and the lead and zinc, so that the cause of death is eliminated. There was no sign of the respiratory distress evident when lead and zinc are present.

During the winter of 1937–8, Evans (Anon. 1937) verified the improved condition of the river. The period of his work followed an unusually wet summer, when the effects of pollution by flood-waters would be expected to be at a maximum. He found that the healthy condition observed by other workers in 1932 was maintained.

A preliminary investigation of the bacterial content of the river water was made by Jones (Anon. 1937), who demonstrated an increase of bacteria in the river with higher temperatures and a decrease with lower temperatures. Though lead is considered to be inimical to the growth of bacteria, she found that the bacterial content of the water was what might be expected in the absence of lead, thus confirming the improved condition of the river water.

Davies (Anon. 1938) investigated the toxicity of dilute solutions of zinc sulphate to aquatic animals and found the following concentrations of zinc to be the highest in which the following animals could live:

	p.p.m.
Pond snail (*Limnaea pereger*) } Fresh-water limpet (*Ancylastrum fluviatile*) }	0·2
Fresh-water shrimp (*Gammarus pulex*)	0·3
Mayfly nymph (*Chloeon simile*)	0·2–0·5
Planarian (*Polycelis nigra*)	30
Water-boatman, stonefly nymph, dragonfly larvae and trichopteran larvae	500

Jones (1940*b*) studied the fauna of the Melindwr, the tributary stream of the Rheidol in which Reese's (1937) botanical experiments had been conducted. He found pollution up to 3 p.p.m., and verified Reese's observation that the river bed was thickly carpeted with gritty mine-dump material. After sampling the fauna above and below the source of pollution by the Goginan dump, Jones (1940*b*) concluded that the fauna of the portion of the river adjoining the mine comprised insect species only. Certain case-carrying trichopteran larvae were absent or infrequent, though carnivorous trichopteran larvae were present: the absence or scarcity of case-carrying larvae was attributed to the absence of vegetable food. This result is of interest in relation to Reese's observations on the river and is a modification of Carpenter's opinion (1926*a*) that the solid detritus from the mines has no deleterious effects upon the fauna.

The results of these investigations show that the Rheidol is recovering from the effects of metallic pollution, and that the fauna and flora of the river are increasing both in species and individuals.

In 1941 emergency conditions led the area to be considered as a source of zinc ores, but no action was taken. The temporary utilization of material from the Cwm Rheidol mine as a source of iron oxide was of short duration, and a projected brick-making scheme at the same mine failed to materialize.

Conclusions

During the past 20 years much scientific work has been done on Cardiganshire streams, especially on the Rheidol. The effects of lead and zinc on plant and animal life have been elucidated and the following conclusions reached:

1. By the weathering of the surface of old dumps and slime pits, galena and zincblende are oxidized to their sulphates which are dissolved by surface water, and carried into rivers.

2. The presence of zinc is as serious a pollution as the presence of lead, and in the case of plants, the former is more deleterious. If plants are grown in the presence of from 1 to 10 p.p.m. zinc, the toxicity increases sharply with the concentration. Since, for chemical reasons, the amount of lead present in the soil water of the dumps and in the rivers is unlikely to be greater than 3 p.p.m., the greater toxicity of zinc is one of the primary causes of the paucity of vegetation on the mine detritus and in the rivers.

3. The coarser material partly composing the dumps is hard and weathers very slowly, and is thus unable to support vegetation.

4. The lack of colonization by plants of the Goginan dump, including the marginal experimental area, is partly due to the blown material from old slime pits which accumulates around the vegetation during and after easterly winds.

5. Phosphate deficiency is probably an accessory cause of the scant vegetation on mine dumps.

6. The addition of lime and phosphate would be beneficial to the soil in the areas of the dumps where other conditions render colonization possible.

7. The presence of detritus from the mines, thickly covering the river beds, mechanically deters the establishment of macrophytes and reduces the microflora. The combined effects of these result in the absence of case-carrying trichopteran larvae (caddis-fly larvae).

8. A sequence was established for the disappearance of the fauna of a polluted stream: a river which is recovering may be expected to pass through the same faunistic and vegetational phases in reverse order. The same phases may be traced in passing downstream from the source of pollution.

9. *Ancylus fluviatilis* among animals, and *Ranunculus aquatilis* among plants, are very sensitive to

the presence of lead and zinc, and are the first to disappear if traces of lead or zinc are present.

10. The cause of the death of fish is not the presence of fragments of galena grit producing clogging and inflammation of the gills, but the formation of an adsorption complex of the heavy metals upon the gills, together with an excess secretion of mucus, causing suffocation, following acute respiratory distress.

11. Settling pits do not obviate pollution, because their use cannot entirely prevent a solution greater than 0·1 p.p.m. lead, and 0·3 p.p.m. zinc, from reaching the rivers. Such solutions are toxic to certain fish.

12. Where galena and blende are mixed, precipitation of suspended matter should be provided for and must be followed by passage of the effluent through a peat filter to remove the colloids.

13. *Towy*. This river is not polluted, but would be in danger from the Nant-y-mwyn mine if any break in the retaining structure occurred.

14. *Dovey*. Trout occur from Llanbrynmair to the sea, and salmon are caught in the lower reaches of the river. The Twymyn is polluted, but its volume is not sufficiently great to cause harm to the main river, which receives a considerable volume of water from other tributaries.

15. *Teify*. Intermittent pollution of the headwaters occurs, but Tregaron Bog, which occupies the site of an old glacial lake, acts as a natural adsorption filter, and cleanses the river of metallic pollution as it meanders through the bog.

16. *Clarach*. The Nant Silo tributary is polluted, but the dilution of the main river by other unpolluted tributaries results in the presence of a normal invertebrate and vertebrate fauna, including trout, in the lower reaches.

17. *Ystwyth*. This is a polluted stream, especially in regard to zinc, and there seems to be no practical remedy at present.

18. *Rheidol*. Extensive surveys were made from chemical, botanical and zoological standpoints. The river affords an interesting study of biological change consequent upon the closure of mines, and of gradual slow recovery, despite periodic setbacks. The main river has now almost fully recovered in its lower reaches, though the Cwm Rheidol and Goginan mines constitute intermittent sources of pollution by lead and zinc. The Goginan mine, in particular, contributes detritus to the stream in flood-time, which is an additional reason for the scanty fauna and flora in that vicinity.

This work was carried out with the aid of grants from the Department of Scientific and Industrial Research, the Ministry of Agriculture and Fisheries, local County Councils, and private individuals. To all of them grateful acknowledgement is made. The investigations were first organized by the River Teify Sub-Committee of the Ministry of Agriculture and Fisheries Standing Committee on Rivers Pollution. In 1924, the Sub-Committee was augmented, and became the Cardiganshire Rivers Pollution Sub-Committee. In 1929, on account of suspected pollution in the Dovey and Towy, the Committee extended the scope of its work, and became the West Wales Rivers Pollution Sub-Committee, which title it now holds. Its Secretary throughout was Lieut. L. H. Milne, the District Inspector of the Fisheries Board of the Ministry of Agriculture and Fisheries, whose ever-ready help and co-operation are gratefully acknowledged by all the workers, together with the help of Dr E. C. Gee, especially in the earlier stages of the investigation.

In conclusion, the author wishes to thank Prof. T. C. James and Prof. R. D. Laurie for their help in the preparation of this paper, and Mr Emrys Bowen for kindly preparing the map. Grateful thanks are also expressed to Mr James Angus, Prof. J. E. Coates, and all those who contributed documents, and thus made it possible for the results to be collated.

REFERENCES

ANON. (1874). *Rep. of Rivers Pollution Commission.*

—— (1923). Minutes of River Teify Sub-Comm. of Minist. Agric. and Fish. Stand. Comm. on Riv. Poll.

—— (1937). Minutes of West Wales Riv. Poll. Sub-Comm. of Minist. Agric. and Fish. Stand. Comm. on Riv. Poll.

—— (1938). Ditto.

—— (1942). Ditto.

CARPENTER, K. E. (1924). The freshwater fauna of the Aberystwyth district of Cardiganshire, studied with especial reference to the pollution of streams consequent on lead-mining operations. No. 49, File F.G. 1898, Minist. Agric. and Fish. Stand. Comm. on Riv. Poll. Also: A study of the fauna of rivers polluted by lead mining in the Aberystwyth district of Cardiganshire. *Ann. appl. Biol.* **11**, 1.

CARPENTER, K. E. (1925). Biological factors involved in destruction of river fisheries, by pollution due to lead mining. Ser. No. 84, Rep. No. 77, Minist. Agric. and Fish. Stand. Comm. on Riv. Poll. Also: *Ann. appl. Biol.* **12**, 1.

—— (1926*a*). The lead mine as an active agent in river pollution. *Ann. appl. Biol.* **13**, 395.

—— (1926*b*). Report on the lethal action of lead salts on fishes. Ser. No. 190, Rep. No. 129, Ref. File F.G. 1655, Minist. Agric. and Fish. Stand. Comm. on Riv. Poll. Also: *Brit. J. exp. Biol.* **4**, 378.

COATES, J. E. & HINKEL, L. E. (1925). Report on the River Teify and its tributary, the Bargoed Brook. Ser. No. 96, Rep. No. 86, Minist. Agric. and Fish. Stand. Comm. on Riv. Poll.

DAVIES, G. N. (1941). An investigation of the effect of zinc sulphate on plants. *Ann. appl. Biol.* **28**, 81.

GRIFFITH, J. H. S. & ANGUS, J. (1917). *The Possible Origin of Pollution from Lead Mines of Cardiganshire.* Cardigan.

GRIFFITH, J. J. (1919). Influence of mines upon land and livestock in Cardiganshire. *J. agric. Sci.* **9**, 366.

HOOPER, M. C. (1937). An investigation of the effects of lead on plants. *Ann. appl. Biol.* **24**, 690.

HOOPER, M. C. & NEWTON, L. (1935). The effect of lead salts on plants in culture, together with some suggestions for the colonisation of the Goginan mine-dump. Ser. No. 733, Rep. No. 517, Off. Ref. 15,878, Minist. Agric. and Fish. Stand. Comm. on Riv. Poll.

JAMES, T. C. (1927). Proceedings of the Technical Advisory Committee of Cardiganshire Rivers Sub-Committee of Minist. Agric. and Fish. Stand. Comm. on Riv. Poll. Ser. No. 232.

JAMES, T. C. & HUDLESTON, L. (1931). Minutes of West Wales Riv. Poll. Sub-Comm. of Minist. Agric. and Fish. Stand. Comm. on Riv. Poll.

JAMES, T. C. & JONES, R. (1927). Report on experiments carried out at the University College of Wales, Aberystwyth, on the removal of traces of metal from solution. Ser. No. 219, Rep. No. 147, Ref. File F.G. 1655A, Minist. Agric. and Fish. Stand. Comm. on Riv. Poll.

—— —— (1928). Result of a field experiment designed to purify a lead mine effluent and of experiments on removal of traces of soluble lead and zinc from mine effluents. Ser. No. 301, Rep. No. 196, Ref. File 1655A, Minist. Agric. and Fish. Stand. Comm. on Riv. Poll.

JAMES, T. C. & LAURIE, R. D. (1931). River Teify. Ser. No. 485, Rep. No. 326, Off. Ref. F.G. 11,215, Minist. Agric. and Fish. Stand. Comm. on Riv. Poll.

JAMES, T. C., LAURIE, R. D. & NEWTON, L. (1932). A study of the present condition of the River Rheidol. Ser. No. 607, Rep. No. 419, Minist. Agric. and Fish. Stand. Comm. on Riv. Poll.

JONES, J. R. E. (1936). The toxicity of dissolved lead salts to the stickleback and other aquatic species in relation to the pollution of the River Rheidol. Ser. No. 746, Rep. No. 527, Off. Ref. F.G. 16,953, Minist. Agric. and Fish. Stand. Comm. on Riv. Poll.

—— (1937). The toxicity of dissolved salts of lead, copper and zinc to the stickleback. Ser. No. 777, Ref. No. 550, Off. Ref. F.G. 18,041, Minutes of West Wales Riv. Poll. Sub-Comm. of Minist. Agric. and Fish. Stand. Comm. on Riv. Poll.

—— (1938). The relative toxicity of salts of lead, zinc and copper to the stickleback (*Gasterosteus aculeatus* L.) and the effect of calcium on the toxicity of lead and zinc salts. *J. exp. Biol.* **15**, 394.

—— (1940*a*). A study of the zinc-polluted River Ystwyth, in North Cardiganshire, Wales. *Ann. appl. Biol.* **27**, 368.

JONES, J. R. E. (1940*b*). The fauna of the River Melindwr, a lead-polluted tributary of the River Rheidol, in North Cardiganshire, Wales. *J. Anim. Ecol.* **9**, 188.

—— (1941). The fauna of the River Dovey, West Wales. *J. Anim. Ecol.* **10**, 12.

JONES, O. T. (1922). Lead and zinc. The mining district of North Cardiganshire and West Montgomeryshire. *Spec. Rep. Minist. Res. G.B.* no. 20.

LAURIE, R. D. & JAMES, T. C. (1931). On the biological examination of the River Twymyn. Ser. No. 483, Rep. No. 324, Off. Ref. F.G. 9294.

LAURIE, R. D. & JONES, J. R. E. (1938). The faunistic recovery of a lead-polluted river in North Cardiganshire, Wales. *J. Anim. Ecol.* **7**, 272.

NEWTON, L. & GLENCROSS, A. (1940). Report on work for session 1939–40. Ser. No. 820, Rep. No. 584, Off. Ref. F.G. 17,572A, Minutes of West Wales Riv. Poll. Sub-Comm. of Minist. Agric. and Fish. Stand. Comm. on Riv. Poll.

NEWTON, L., DAVIES, G. N. & JONES, H. (1938). Report on work in botany Department 1937–38. Ser. No. 809, Rep. No. 577, Off. Ref. 17,572A, Minutes of West Wales Riv. Poll. Sub-Comm. of Minist. Agric. and Fish. Stand. Comm. on Riv. Poll.

NEWTON, L., REESE, M. J. & DAVIES, G. N. (1937). Interim report on botanical work on rivers Rheidol and Melindwr. Ser. No. 771, Rep. No. 545, Off. Ref. F.G. 17,955, Minutes of West Wales Riv. Poll. Sub-Comm. of Minist. Agric. and Fish. Stand. Comm. on Riv. Poll.

NEWTON, L., REESE, M. J. & HOOPER, M. C. (1936). Report on work during the session 1935–36 on the rivers Rheidol and Melindwr. Ser. No. 755, Rep. No. 534, Off. Ref. F.G. 15,780, Minutes of West Wales Riv. Poll. Sub-Comm. of Minist. Agric. and Fish. Stand. Comm. on Riv. Poll.

ORTMANN, A. E. (1909). On the destruction of the freshwater fauna in W. Pennsylvania. *Proc. Amer. phil. Soc.* **48**, 90.

PURDUM, R. B. & RUTHERFORD, H. A. (1933). The solubilities of sparingly soluble salts, using large volumes of solvents. 1. The solubility of lead sulphate. *J. Amer. chem. Soc.* **55**, 3221.

REESE, M. J. (1937). The microflora of the non-calcareous streams Rheidol and Melindwr, with special reference to water pollution from lead mines in Cardiganshire. *J. Ecol.* **25**, 385.

ROBERTS, C. H. & LEACH, T. S. (1929). Treatment of lead mine effluents. Ser. No. 366, Rep. No. 238, Off. Ref. 2947, Minist. Agric. and Fish. Stand. Comm. on Riv. Poll.

(*Received* 2 *April* 1943)

Physiological cotton wilt in the Sudan Gezira

By A. S. Boughey, B.Sc., Ph.D., *Plant Pathologist, Agricultural Research Institute, Anglo-Egyptian Sudan*

(With Plate 1 and 5 Text-figures)

Cotton wilt of physiological origin appears to have been present in the Gezira area of the Anglo-Egyptian Sudan since 1924 if not previously. The present investigations showed that between mid-October and mid-December three factors occur which cause water stress in the crop. At some time during this period there is marked reduction in the size of the absorbing system of the plant, correlated with maximum boll development. Greatly increased day temperatures and higher evaporation prevail, while during the whole period available water in the soil declines. The hypothesis is put forward that when all three factors occur at the same time at their maximum intensity, permanent wilting and death of the plants result; at lesser intensities, wilting is followed by recovery, but with reduction of yield. Experiments are described, the results of which appear to confirm this view. In the author's opinion a return to the earlier sowing date formerly favoured locally would lead to considerably increased losses.

Introduction

Massey (1935) first referred to a serious attack of cotton wilt in the Sudan Gezira, although it is stated to have been noticed in late October for at least 10 years previously.

Symptoms of the wilt, other than the initial wilting, are variable, but in degree rather than kind. The most remarkable feature is the absence of obvious damage to the tap root and main laterals. Plants severely attacked appear to have died suddenly, shrivelled leaves and buds remaining attached to the dead, but still outstanding, petioles and branches. Moderate attacks may cause the shedding of all but the youngest leaves and more mature bolls, or merely result in the death of shoot tips, and the shedding of a few lower leaves and young buds. Recovery is possible in all these stages, even when all the above-ground portion of the plant is apparently dead. Wilt may occur from the 10th week after sowing onwards. Normally, maximum attack takes place between the 12th and 20th weeks, i.e. in November or December.

Andrews & Clouston (1937) calculated that plants wilting moderately but recovering suffered a 20% loss of yield. The average plant mortality over large areas has never exceeded 2·5%, but complete loss of crop from small plots (1–3 acres) is common.

The distribution of wilt is more affected by local soil variations than experimental treatments, low-lying areas being the worst affected (Andrews & Clouston, 1937). There is also a positive correlation between incidence of wilt and the alkalinity of the top 2 ft. of soil (Greene, 1934, 1935). Increasing the number of plants per hole, close spacing and fallowing all decrease wilt, while nitrogen manuring, cropping, and light watering increase it; varietal differences in susceptibility to wilt exist (Andrews & Clouston, 1937, 1938).

Various explanations of the occurrence of wilt have been put forward. Massey (1935, 1936) considered that when soil aeration was bad, and alkali content high, a severe rotting of the finest rootlets, accompanied by fungal attack, occurred, leading in severe cases to wilting and death of the plant. Butler (1938) suggested that there was an accumulation of toxins in the impermeable soil layer (at 18–24 in. depth), which damaged the apex of the root system and caused wilt. Recovery occurred when the roots passed beyond this layer. Clouston (? 1943) stated that physiological rotting of the secondary rootlets occurred in the impermeable soil layer, where replacement of oxygen and removal of harmful root excretions were slow. Under certain conditions *Rhizoctonia* sp. increased in virulence or numbers, invaded these physiologically rotted portions, and attacked the tap root until wilting occurred.

None of these theories as to the cause of wilt is tenable (Anon. ? 1943). No single fungus, or combination of fungi, isolated from cotton roots, produced symptoms of wilt when inoculated into healthy or damaged roots of mature cotton. Plants grown in soil, or watered with soil extracts from the impermeable layer, gave no evidence of the presence there of toxic substances. Cotton grown in drums of soil, which were completely sealed, or sealed and carbon dioxide passed through, did not succumb to wilt, or show marked rotting of the roots.

The work to be described was undertaken to determine the precise nature of cotton wilt, and whether its recent absence from the Gezira is to be regarded as temporary or permanent. The investigation is based on the detailed study of root growth.

Root investigations

(*a*) *Experimental methods*

Observations were made during the 1941–2 season on a 2½-acre plot of Massey's Selected Domains Sakel. The plot was on an internal three-course cotton,

Dolichos lablab, fallow rotation, clean weeded at all times, and laid out in randomized blocks with four replications. It was irrigated when necessary until the end of the rainy season in October, after which it was watered fortnightly throughout the season. Cotton was sown on 15 Aug. Irrigation ceased at the end of March.

Root growth was followed, employing fortnightly excavations (Andrews & Clouston, 1938), in which a rectangular area of soil surrounding a given number of plants in a uniform stand is excavated in steps. The roots in each step are washed from the soil and separated into 'fine' and 'secondary' roots, according to the presence or absence of cork formation in the cortex. The dry weight of fine roots is taken to be a measure of the potential absorbing capacity of the root system.

Fortnightly records were also made of shoot growth, comprising stem height and weight, extent of leaf-shedding, weight of leaves, and number and weight of buds, bolls, and flowers.

The soil-moisture content, in 6 in. steps down to 4 ft., was measured on the dry land the day of irrigation, and on the wet land 2 days after, when all standing water had disappeared. A fair estimate was thus obtained of water lost during the irrigation period, and water added during irrigation. The method is inaccurate in that it fails to cover a short period immediately following irrigation, but it is believed that this cannot affect the general conclusions drawn.

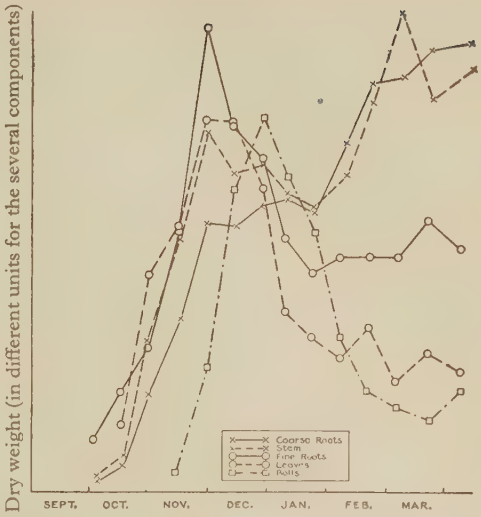
Data on air temperature and evaporation were obtained from a meteorological screen situated near the experimental plot.

(*b*) *Results*

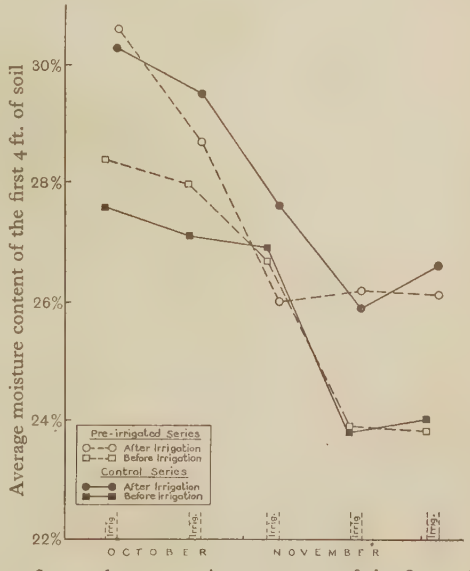
Text-fig. 1 shows the fortnightly variation in dry weight of the several plant components throughout the season. At the end of November boll development becomes very marked and there is a considerable decrease in the weight of leaves and fine roots, while that of stem and secondary roots remains stationary until boll development declines at the end of January.

Text-fig. 2 shows the average soil-moisture content at the beginning and end of the irrigation cycles. Available water is calculated by taking 20% as the wilting point for cotton (which dries out soil to approximately 19·5–20·5%) and 4 ft. as the maximum depth to which roots operate and water penetrates. From a peak period about 8 weeks after sowing, there is a progressive and marked decline in the amount of available water both before and after irrigation, continuing to approximately the 18th week.

Text-fig. 3 shows the recorded values for temperature and evaporation during 1941 and a 10-year average. The highest day temperatures during the cotton season occur from about mid-October to



Text-fig. 1. Development of the several components of the Massey's Selected Domains Sakel plant throughout the season.



Text-fig. 2. Average moisture content of the first 4 ft. of soil before and after irrigation.

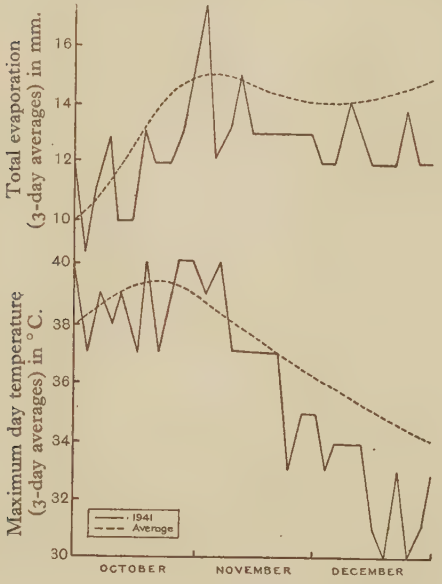
mid-November and coincide with a period of high evaporation. (Evaporation increases again about mid-December and rises throughout the rest of the cotton season to a second and higher peak in April.)

(*c*) *Conclusions*

From these data on plant growth, soil water, and atmospheric conditions, it can be deduced that

between the 10th and 18th weeks after sowing, i.e. mid-October to mid-December, three factors may cause water stress in the crop. At some time during this period there is a drastic reduction in the size of the absorbing system of the plant, correlated with the attainment of maximum boll development. Considerably increased day temperatures, accompanied by higher evaporation, prevail, while during the whole time the amount of available water in the soil declines rapidly.

Within the limits of this 2-month period, the time and intensity of operation of these three factors are variable. Consequently, a coincidence of all three factors at high intensity is possible. It is postulated



Text-fig. 3. 1941, and average, records of maximum air temperature and evaporation.

that when this coincidence occurs, permanent wilting and death of the plants result. A coincidence at lesser intensities leads to permanent wilting, followed by recovery, and is accompanied by some degree of leaf, boll, and bud shedding, with loss of yield.

Various experimental results and records are examined to determine how far this hypothesis agrees with the known facts.

(i) *Resistance to wilt*

The var. Massey's Selected Domains Sakel (M.S.D.S.) in the 1941–2 season reached a peak of maximum fine-root production at the end of November. X1530A, a selection originally from Domains Sakel, matures about 2 weeks later than M.S.D.S., and its maximum fine-root development is similarly delayed. The root system of X1530A does not normally deteriorate until the crop has passed the critical period of high temperature and evaporation, usually occurring mid-October to mid-November. The expectation that X1530A will be less susceptible to wilt than M.S.D.S. is realized (Andrews & Clouston, 1938). If a period of high temperatures occurs about a month later than usual, the position as regards resistance in these two varieties is reversed. Such a late hot spell occurred in 1936 (see Text-fig. 4), and contrary to all previous observations, M.S.D.S. was the more wilt-resistant of the two varieties (Andrews & Clouston, 1938). It may be supposed that the M.S.D.S. had nearly matured its first crop of bolls, and was entering upon a second phase of vegetative growth when this late hot spell occurred. The later maturing X1530A was caught in its most wilt-susceptible stage, when owing to its carrying a heavy crop of bolls its fine-root system was in a moribund condition.

Text-fig. 4. 1936, and average, maximum air temperature.

The scattered distribution of wilted plants through an infected plot is to be expected if wilt is intimately linked with the stage of maturity, since early- and late-flowering plants, and those with a heavy or light crop of bolls, are distributed through the plot at random.

(ii) *The effect of soil treatments on wilt*

During the period necessary to raise a cotton crop Gezira soil suffers a temporary deterioration which is associated with a decreased water permeability, and contributes to the progressive reduction in water penetration as the season advances. In plots under cotton in March, water in quantity may only penetrate the first foot of soil, whereas at the beginning of the season at least 2 ft. is reached.

An experiment was performed in the 1941–2 season to determine what effect an early onset of this

soil deterioration would have on cotton growth and water penetration. To produce the effect the land was given nine fortnightly irrigations before sowing. The results are shown in Tables 1 and 2, and Text-fig. 2.

From mid-October to mid-November the amount of irrigation water taken up by the soil was less in the pre-irrigated than in the control series: the amount of water lost was also less in the pre-irrigated plots

TABLE 1. *Effect of pre-sowing irrigations on cotton leaf-shed and boll number*

C. = control series; P. = pre-irrigated series.

		No. of leaves shed	No. of bolls
8 Nov.	C.	8·8	2·2
	P.	9·5*	2·1
22 Nov.	C.	10·6	7·8
	P.	11·5	7·3
6 Dec.	C.	11·9	11·7
	P.	12·9*	11·5
20 Dec.	C.	14·3	12·7
	P.	14·0	11·0*

* Difference significant at $P = 0{\cdot}05$ level.

TABLE 2. *Effect of pre-sowing irrigations on water loss from the soil*

C. = control series; P. = pre-irrigated series.

Irrigation period ending		Amount of water lost (c.m./feddan)*
20 Oct.	C.	199
	P.	161†
3 Nov.	C.	161
	P.	124†
17 Nov.	C.	236
	P.	130†
1 Dec.	C.	118
	P.	149
15 Dec.	C.	168
	P.	137

* 1 feddan = 4200·833 sq. m. = 5024·17 sq. yd. = 1·038 acres.

† Difference significant at $P = 0{\cdot}05$ level.

(Table 2). As cotton on the plots of both series was of the same size at this time, and temporary wilting occurred in both, it may be assumed that the pre-irrigated series was under greater water-strain, which induced the increased leaf- and boll-shedding shown in Table 1.

In a second experiment on the effect of reduced water permeability (Boughey, ? 1943), surface soil was 'puddled' by boys walking up and down the furrows immediately after irrigation. ('Puddling' reduces the water permeability of Gezira soil.) This 'puddling' was started 6 weeks after sowing, and was continued for four irrigations. Observations were not made on differences in water penetration or leaf-shed, but cotton yield was significantly depressed by the treatment.

Increasing the amount of water in the 3rd and 4th feet by boring holes to these two depths and filling them with fine gravel significantly increased cotton yield (Boughey, ? 1943), which, in this instance, can only be due to an increase in available soil water lessening the extent of leaf, bud, and boll shed.

A similar explanation is possible in experiments on the use of gypsum as a 'soil improver' in the Gezira (Greene, 1934). Addition of gypsum to the soil increased water permeability and cotton yields. Greene & Snow (1939) attributed the increase in yield to increased nitrification.

Summarizing these soil treatments: from the 10th to 18th week after sowing, water stress, leading to temporary wilting at least, normally occurs in the cotton crop. Its alleviation by increasing the amount of available soil water leads to decreased leaf, bud, and boll shedding, and increased yields. Increasing it, by reducing the amount of water, produces increased shedding and decreased yields.

No experiment dealt directly with the effect of increased water supply on permanent wilting, but commercial practice provides evidence on this point. In the commercial cotton-growing area 11- or 12-day interval irrigations during October and November give better results than the standard 14-day interval. Since this practice was adopted together with a somewhat later sowing date (*c.* 20 Aug.) no general wilt attack has been reported from areas under X1730A (a var. similar to X1530A).

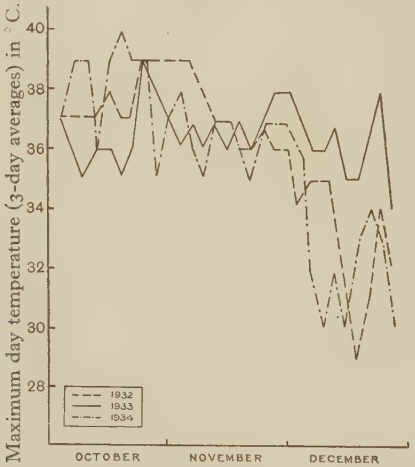
(iii) *The effect of seasonal variations*

Seasonal variation can affect the water relations of the plant by the early destruction of the water permeability of the soil through heavy rain; by varying the length, date, and severity of the 'critical' period of high temperatures and evaporation; or by modifying the size of the plant and delaying or hastening maturation.

Crowther & Crowther (1935) showed that early (Apr.–June) rain depresses yield, and Crowther (1934, 1935) demonstrated that protecting plants from early rain increases yield. The general explanation of this effect of early rain is that the water permeability of the soil is affected, although opinions differ as to the manner in which it is affected. Both premature closure of the soil cracks (Greene, 1934, 1935), and their filling with impermeable soil (Jewitt, 1941), have been held responsible. It is clear that a reduction in water permeability of the soil through heavy early rain may be a factor in reducing yield.

The effect on the crop of high temperatures during October and November has not been sufficiently stressed, for the amount of wilting, and final yield, are closely correlated with the maximum day temperature at this time.

In 1933–4 mortality from wilt was high. The early rains in 1933 were abnormally heavy and there was an unusually hot spell (see Text-fig. 5) towards the end of November, lasting throughout December. No data on root growth are available for this season, but it may be supposed that at the time of this hot spell the cotton was maturing its first crop of bolls. The fine-root system would then be in poor condition and unable to meet the extra strain placed upon it during this period. The crop of bolls would be too far advanced to be shed, and no recovery from wilt would be possible. The previous season had also suffered heavy early rains and an abnormally hot spell. The latter occurred much earlier (see Text-fig. 5), when the crop would not be carrying many mature bolls. No mortality from wilt was recorded,



Text-fig. 5. 1932–34 maximum air temperatures.

though it was in 1933–4, because, in the absence of mature bolls, the plant could shed all young buds and bolls, and enter a renewed phase of vegetative growth with vigorous root development.

Table 3 shows the relationship between yield, maximum day temperatures, and early rainfall. Cotton yields are from the main crop Domains Sakel at the Gezira Research Farm. The figures under temperature indicate the number of days above average temperature during the 'critical' period, counting only occasions of four or more of such consecutive days. Only seasons from 1934 to 1935 onwards are considered, since previously blackarm (*Xanthomonas malvacearum*) and leaf curl had not been adequately controlled, and their effect on yield complicates the issue.

Table 3 shows that seasons of hot spells during the 'critical' period in Oct.–Nov. have lower yields. There is no evidence that early rainfall has any specific effect, for the records are few, but clearly a heavy early rain does not prevent a high yield if the weather is cool.

(iv) *Out of season sowing experiments*

A second period of increasing temperatures and evaporation, somewhat similar to, but more severe than, that of Oct.–Nov., begins in April. Cotton sown out of season in December, and maturing in April, suffers severely from wilt attack as compared with cotton sown in Sept.–Nov. The symptoms produced are identical with those of wilt at the normal time, and leave little doubt that it is caused by the effect of high temperatures on plants whose water uptake is reduced through the curtailment of growth of the root system.

(v) *De-budding experiments*

De-budded plants develop a larger fine-root system (Andrews & Clouston, 1937). They are not attacked by wilt, and give increased yields, which in 1932–3 reached significance (Crowther, 1934).

Table 3. *Effect of maximum day temperatures and early rainfall on cotton yields at Gezira Research Farm*

Season	Yield (kg./feddan)	Days of high temp. during 'critical' period	Amount of early rain (cm.) above av. for period
1937–38	4·83	0	+20
1938–39	4·17	0	−59
1934–35	4·07	0	+10
1939–40	3·85	0	+32
1936–37	3·79	22	+14
1941–42	3·50	12	−36
1935–36	3·23	9	+ 4
1940–41	3·02	9	+14

In 1932 a fairly severe early attack of wilt must have occurred (see the temperatures in Text-fig. 5). The delay in maturation due to de-budding would therefore enable the plants to avoid this unfavourable period and the consequent shedding of leaves, buds and bolls. In the following two seasons, when yield increases were not significant, the weather during the 'critical' period was less unfavourable.

(vi) *Ringing experiments*

Plants ringed down to the wood at soil level, after irrigation in late October, usually start to wilt, and have shed some lower leaves and buds before the next irrigation. Temporary recovery takes place on watering, but wilting, with heavy leaf- and bud-shed, and usually death, occur again before the second irrigation. Such plants have a reduced root system in a poor condition, with much rotting and fungal invasion, i.e. ringing reproduces the exact symptoms of severe wilt attack.

Discussion

Considerable evidence is available to support the hypothesis that cotton wilt in the Gezira is produced by abnormally high temperatures, operating during

a period of high evaporation, on plants whose water uptake has been reduced, through the temporary cessation of root growth and a reduction in the amount of water available in the soil. Should these conditions occur late, when the plant is bearing a fair number of maturing bolls which will not be shed, there is widespread plant mortality. An earlier occurrence, when the majority of the bolls can still be shed, causes a period of prolonged wilting, followed by recovery. In all cases there is some degree of bud, boll, and leaf-shed, accompanied by loss of yield.

Physiological bud-shedding, i.e. bud-shedding which is not the result of pest or disease attack, clearly arises in the Gezira from two causes: severe water strain, and nutrient deficiency. Shedding from the latter cause is omnipresent and is a normal accompaniment of the maturation processes of the plant. The former occurs only under wilt conditions, and generally precedes the second. The roots, owing to their position at the base of the carbohydrate gradient in the plant, will suffer first from any deflexion of carbohydrates to the developing bolls, while nitrogen starvation commences with the general cessation of root growth through lack of carbohydrates. If the plant is to lose buds through the effect of water-strain, this will usually happen before the deflexion of carbohydrates and nitrates to the developing bolls becomes sufficiently intense to cause bud-shed. Loss of yield after wilt attack is induced not so much by the loss of buds, bolls, and growing points, as by the increased leaf-shed, which was shown to have serious effects on yield (Crowther, 1941). The shedding of buds is beneficial in that it prevents the continued deflexion of food supplies and makes possible a renewal of root activity, with a consequent relief of water-strain and nitrogen deficiency. The crop will have been delayed, and it may not be possible to mature sufficient numbers of bolls to compensate for the time and material lost. There must also be small losses from a general reduction in photosynthetic activity during wilting, for the high leaf temperatures which result when the leaf is losing insufficient water to keep itself cool affect the efficiency of the chlorophyll system, in addition to setting in operation the abscission mechanism which (Balls, 1919) brings about premature leaf-shed in the older leaves.

A satisfactory explanation is thus afforded for the recorded effects of various treatments on wilt.

Nitrogenous manuring, which increases wilt (Andrews & Clouston, 1937, 1938), will increase the shoot/root ratio, so predisposing the plant to water-strain. Wide spacing, and decreasing the number of plants per hole, which increase the amount of nitrogen available per plant, have a similar effect, which cannot be compensated for by the extra amount of water available, since the shoot/root ratio will again be less favourable.

The fact that plants in the poorer soil of the Northern Gezira are small, and have a lower shoot/root ratio, explains why Domains Sakel can be grown there without serious losses from wilt. Nitrogenous manurial trials there, and on poor land in the west on the White Nile, have not invariably given an increase in yield.

High soil alkalinity is correlated with wilt (Greene, 1934, 1935) because it is associated with a lower soil-water permeability.

There are two stages of wilt, the one causing plant mortality, the other permanent wilting, but not mortality. The later stage, which causes plant mortality, is obvious, but the earlier permanent wilting stage grades imperceptibly into the temporary mid-day wilting, so prominent a feature of the Gezira cotton during October and November, and is easily overlooked.

Temporary wilting at mid-day, during the hottest weather in October and November, occurs with every cotton variety towards the end of an irrigation period in the Gezira. There is no over-heating of leaves to be noticed however, if they are able to recover their turgidity overnight. It is only when water-strain is so great that the crop is unable to recover complete turgidity overnight that over-heating of leaves occurs at mid-day. When this happens the plant is suffering from an attack of wilt, and will lose some of its leaves, buds, or bolls.

Leaf-shed from this cause is commonly ascribed, somewhat vaguely, to 'maturity', and losses in yield from this early phase of wilt attack have therefore been insufficiently stressed. In part, they may account for the recorded depressions in Gezira cotton yields for which no satisfactory explanation has as yet been put forward. The depressing effect of early rain on yield (Crowther & Crowther, 1935), which is a composite effect, will include losses from wilt. The significance of the wilt losses will not, however, be properly represented, for the temperature factor, the more important environmental factor, is not taken into consideration. (Much of the depressing effect of early rains on yield was due to the fact that the incidence of leaf curl and black-arm is positively correlated with the amount of early rain. Since these two diseases were properly controlled the early rains-yield correlation has broken down.)

Wilt can be controlled by adjusting the maturation period of the plant with regard to the Oct.–Nov. 'critical' period of high day temperatures, or increasing the amount of water available to the plant during this period. The first is achieved by delaying the sowing date, or using a later maturing cotton variety; the second by increasing the frequency and amount of irrigation.

The present disappearance of wilt from the southern and central Gezira must be regarded as a temporary condition, the result of delaying the

sowing date till *c.* 20 Aug., planting the variety X1730A, which matures a fortnight later than the Domains Sakel it replaced, and the use of an 11–12-day irrigation interval in October and November.

The delayed sowing date was adopted as a blackarm control measure. As blackarm-resistant cotton varieties become available for general planting, it will again be possible to consider the adoption of an earlier sowing date, as there are strong practical reasons in favour of such a course. In this event it is believed that wilt losses will once more become serious, unless greater root vigour can be incorporated in these varieties, for no appreciable change can be made at present in the irrigation interval.

REFERENCES

Andrews, F. W. & Clouston, T. W. (1937). Section of Botany and Plant Pathology. Agricultural Research Service. *Rep. Dep. agric. For., Sudan Govt.* 1936, pp. 33, 34.

—— —— (1938). Section of Botany and Plant Pathology. Agricultural Research Service. *Rep. Dep. agric. For., Sudan Govt.* 1937, pp. 36, 42.

Anon. [? 1943]. *Annual Reports of the Agricultural Research Service, Sudan Government,* 1938–9 and 1939–40 (in the Press).

Balls, W. L. (1919). *The Cotton Plant in Egypt.*

Boughey, A. S. [? 1943]. *Report of the Agricultural Research Institute, Sudan Government,* 1940 (in the Press).

Butler, Sir Edwin J. (1938). Memorandum on cotton wilt in the Sudan Gezira [mimeographed, unpublished].

Clouston, T. W. [? 1943]. *Report of the Agricultural Research Institute, Sudan Government,* 1940 (in the Press).

Crowther, E. M. & Crowther, F. (1935). Rainfall and cotton yields in the Sudan Gezira. *Proc. roy. Soc.* B, **118**, 343.

Crowther, F. (1934). Plant Physiology Section, Gezira Agricultural Research Scheme. Final Report on Experimental Work, 1932–33. *Rep. Gezira agric. Res. Serv., Sudan Govt.* 1933, p. 73.

—— (1935). Plant Physiology Section, Gezira Agricultural Research Scheme. Report by Mr F. Crowther on experimental work carried out by the staff of the section during the season 1933–34. *Rep. Gezira agric. Res. Serv., Sudan Govt.* 1934, p. 41.

Crowther, F. (1941). Studies in growth analysis of the cotton plant under irrigation in the Sudan. II. Seasonal variation in development and yield. *Ann. Bot., Lond.,* N.S. **5**, 509.

Greene, H. (1934). Gezira Chemical Section, Gezira Agricultural Research Scheme. Final Report on Experimental Work in 1932–33. *Rep. Gezira agric. Res. Serv., Sudan Govt.* 1933, p. 92.

—— (1935). Gezira Chemical Section, Gezira Agricultural Research Scheme. Report by Dr H. Greene on experimental work carried out by the staff of the section in 1933–34. *Rep. Gezira agric. Res. Serv., Sudan Govt.* 1934, pp. 82, 93.

Greene, H. & Snow, O. W. (1939). Soil improvement in the Sudan Gezira. *J. agric. Sci.* **29**, 1.

Jewitt, T. N. (1941). Dispersion studies on Gezira soil. *J. agric. Sci.* **31**, 466.

Massey, R. E. (1935). Section of Botany and Plant Pathology, Gezira Agricultural Research Scheme. Report by Mr Massey on experimental work carried out by the staff of the section during season 1933–34. *Rep. Gezira agric. Res. Serv., Sudan Govt.* 1934, p. 124.

—— (1936). Section of Botany and Plant Pathology, Agricultural Research Service. Report by Mr R. E. Massey on experimental work carried out by the staff of the section during season 1934–35. *Rep. Gezira agric. Res. Serv., Sudan Govt.* 1935, p. 53.

EXPLANATION OF PLATE 1

Fig. 1. Method of excavating cotton roots.

Fig. 2. Plants showing temporary wilting at mid-day just before irrigation in late October.

Fig. 3. Close up of a permanently wilting plant in early November, at mid-day just prior to irrigation. The older lower leaves have been killed and will be shed, the younger upper leaves are hot to the touch, but recover their turgidity after irrigation.

Figs. 4 and 5. Furrows of adjacent subplots 5 days after irrigation. Owing to watering difficulties the subplot in Fig. 4 was one day late for its irrigation during a hot spell in early November, when the cotton was wilting. Note the difference in leaf-shed between this cotton and that in the adjacent subplot shown in Fig. 5.

(*Received* 15 *April* 1943)

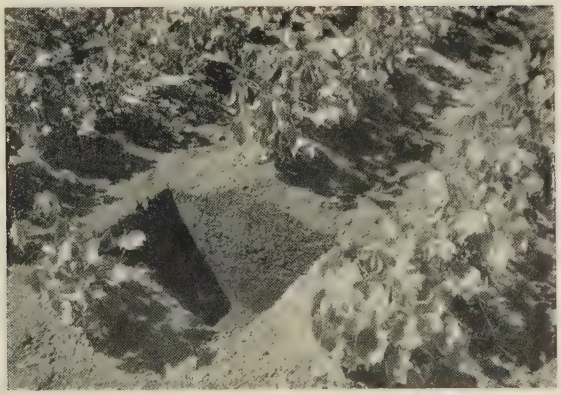


Fig. 1

Fig. 2

Fig. 3

Fig. 4

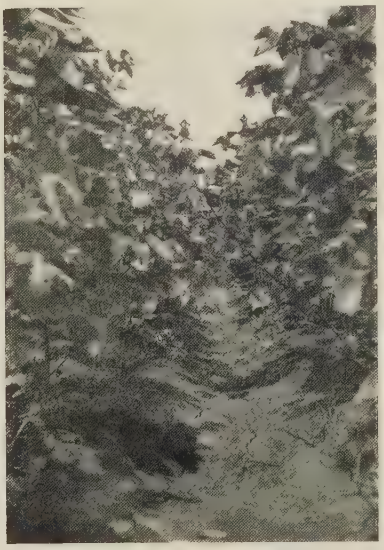


Fig. 5

The nutrition of the pea

By R. M. Woodman, *Horticultural Research Station, School of Agriculture, Cambridge University*

Sand-culture experiments with the pea plant demonstrated that 8·24–32·96 p.p.m. of available nitrogen was the optimum range of concentrations for yield of peas, with a bias in favour of the lower half of the range; adequate supplies of nitrogen appeared to cause branching of the tops into two or more main stems. The best range of concentrations of available phosphorus was 2·73–10·92 p.p.m., while that for available potassium was 5·61–44·88 p.p.m. Deficiency of potassium caused marginal scorch followed by general scorch and death of the foliage progressively up the stem. Absence of boron had not a great effect with this plant, probably because the large seed possibly contains an appreciable amount of boron.

Methods have been discussed (Woodman, 1940). The variety of pea was Lincoln, from Messrs Charles Sharpe & Co. The concentrations in p.p.m. of the important elements in the nitrogen and phosphorus experiments have been given (Woodman, 1942, Table 1). Concentrations of the element particularly concerned are: *Nitrogen experiment*: nitrogen, solution A, 65·92; B, 32·96; C, 24·72; D, 16·48; E, 8·24; F, 4·12; and G, 0·41. *Phosphorus experiment*: phosphorus, solution A, 43·68; B, 21·84; C, 16·38; D, 10·92; E, 5·46; F, 2·73; and G, 0·27. The concentrations of the elements present in the *potassium and boron experiments* are given in Table 1 of this communication, variations being italicized. In all the experiments, the concentrations of the minor and other elements present were as those given (Woodman, 1942, Table 1).

Table 1. *Concentrations in p.p.m. of the important elements present in the potassium and boron experiments*

Solution	N	P	K	B	S	Na
A	20·60	8·19	*44·88*	0·0681	*25·64*	46·00
B	20·60	8·19	22·44	*Nil*	16·44	46·00
C	20·60	8·19	*22·44*	0·0681	*16·44*	46·00
D	20·60	8·19	*11·22*	0·0681	*11·84*	46·00
E	20·60	8·19	*5·61*	0·0681	*9·54*	46·00
F	20·60	8·19	*2·81*	0·0681	*8·39*	46·00
G	20·60	8·19	*0·28*	0·0681	*7·36*	46·00

Nitrogen experiment

The 112 waxed plant-pots were arranged in 16 replications of a randomized block of the 7 treatments A–G. Seed sown, 26 Mar. 1941; germinated by 1 Apr.; seedlings singled to one per pot, 6 Apr.; and cultures harvested, 30 June 1941. Each culture received 11·5 l. of its appropriate solution in lots of 250 c.c. about three times weekly.

By harvest, the tops with most nitrogen were drying off. They were branched from near the sand surface, sometimes into more than two main stems; and on many cultures subsidiary shoots about 6 in. long were present. The roots with treatment A were relatively small, and the pods small and often empty, while the peas were small and immature. Solution B gave better pods and peas; three cultures bore root nodules. Tops with C were not so ripe or branched; there were never more than two main stems, and usually only one. The pods were greener and plumper than with A and B. There were root nodules on 10 cultures. The tops with D were greener and still less branched, with plump pods; there were nodules on 10 roots. E gave similar results, the individual peas being the largest in the whole experiment; 13 roots had nodules. Treatment F resulted in smaller plants with one stem only, smaller pods, and nodules on 10 roots. The smallest concentration of nitrogen, G, yielded still smaller cultures with one stem only, and nodules on 3 roots.

On 26 May the numbers of plants with flowers were: A, 4; B, 8; C, 8; D, 12; E, 8; F, 5; and G, 2. By 4 June, every culture with A, B, C, D, E, and F, and 13 with G, had flowers or pods forming.

It was evident that a good supply of nitrogen resulted in a branching top; the branching met with in the experiments to be described later could possibly be ascribed to the adequate amount of nitrogen in the solutions used. It was further apparent that the presence of root nodules must have compensated to some extent for any deficiency in the nitrogen supplied.

Table 2 contains the usual subtables of results. Over a long range of nitrogen, E = 8·24 to B = 32·96 p.p.m., there was no response as regards fresh weight of the whole tops (B = C = D = E). For shelled peas in the fresh state, there was a bias in favour of C, D, and E, with possibly D and E as the best; nitrogen in excess or deficit of this range led to diminution in yield (C = D = E > A = F = G). The dried peas bore this out: B = C = D = E > A = F = G. The optimum range of concentrations of available nitrogen for pea production was therefore E, D, C, and B, 8·24–32·96 p.p.m., with some bias in favour of the lower half of the range.

Yields with G, 0·41 p.p.m. of N, were rather high, and may have been due to one or both of the following factors: nitrogen content of the large seed, and nodules on some of the roots.

The yield of whole tops was relatively favoured by high nitrogen, as was obvious from the two subtables for whole top/root ratios. A deficiency of nitrogen (G) led to an increased moisture content of the shelled peas, and an excess (A) to a decreased one (G > D = E = F > A).

Table 3 contains the summaries of results. The best range of phosphorus for fresh shelled peas was F, E, and D, 2·73–10·92 p.p.m., higher concentrations leading to reduced yields, and serious deficiency to a still greater reduction, D = E = F > A = B = C > G; this was largely borne out by the yields of dried peas. The yields with G, 0·27 p.p.m. only of P, indicated the possibility of appreciable amounts of phosphorus in the large seed.

Whole top/root ratios, and the moisture contents

TABLE 2. *Summaries of results for nitrogen experiment with peas. Weights in g.*

Description of data		Treatment mean for A	B	C	D	E	F	G	Mean of all results	S.E.
Whole tops, FW	SSS	24·08	24·84	31·14	29·34	26·92	11·08	6·33	21·96	2·278
		B = C = D = E > F = G; C > A > F = G; A = B = D = E								
Roots, FW	SS	2·69	2·84	3·35	3·76	3·48	3·46	1·38	2·99	0·442
		B = C = D = E = F > G; A = G; A = B = C = D = E = F								
Whole plants, FW	SSS	26·77	27·67	34·48	33·10	30·40	14·54	7·71	24·95	2·586
		A = B = C = D = E > F = G								
Shelled peas, FW	SSS	2·36	6·79	8·12	8·77	8·62	3·47	2·38	5·79	0·647
		D > B > A = F = G; C = D = E > A = F = G; B = C = E								
Whole tops, DW	SSS	9·39	12·23	12·37	12·53	11·92	4·96	3·29	9·53	0·821
		B = C = D = E > A > F = G								
Roots, DW	NS	0·44	0·57	0·52	0·53	0·46	0·33	0·16	0·43	0·236
Whole plants, DW	SSS	9·83	12·80	12·89	13·06	12·39	5·28	3·45	9·96	0·799
		B = C = D = E > A > F = G								
Shelled peas, DW	SSS	0·77	2·12	2·40	2·62	2·53	1·04	0·72	1·74	0·212
		B = C = D = E > A = F = G								
Whole top/root, FW	SSS	17·73	9·74	10·26	8·94	8·57	4·46	4·66	9·19	1·380
		A > B = C = D = E; B = C = D > F = G; E = F = G								
Whole top/root, DW	SSS	43·16	24·20	25·93	25·50	25·60	16·56	19·32	25·75	3·508
		A > B = C = D = E = F = G								
Whole tops, % moisture	S	55·02	48·65	59·51	56·94	55·87	48·38	52·60	53·85	2·646
		A = C = D = E = G; C = D > B = F; A = B = E = F = G								
Roots, % moisture	SSS	80·91	78·50	83·71	85·07	85·80	87·46	87·99	84·21	1·276
		G > C > B; D = E = F = G > A = B; A = C; C = D = E = F								
Whole plants, % moisture	NS	56·75	51·58	61·76	60·33	59·98	59·32	59·66	58·48	2·500
Shelled peas, % moisture	SSS	67·13	68·65	70·13	70·51	70·81	70·73	77·10	70·72	1·040
		G > D = E = F > A; G > B = C = D = E = F; A = B = C								

PHOSPHORUS EXPERIMENT

The 70 glazed culture jars were arranged in 10 replications of a randomized block of the 7 treatments A–G. Seed sown, 24 Mar. 1941; germinated by 31 Mar.; seedlings singled, 6 Apr.; and cultures harvested, 25 June 1941. Each culture received 18·4 l. of its appropriate solution in lots of 400 c.c. about every 2 days.

At harvest, treatments A, B, C, D, E, and F, gave similar plants with ripening tops and large, bushy roots; there were root nodules on two with treatment D, four with E, and three with F. Pods with D, E, and F were large and plump. G yielded small cultures with one or two pods, usually one, on each. Treatments A–F resulted in branched stems, but G in single ones. By harvest, every plant had flowered.

of the whole tops, roots, and whole plants, did not respond to variations in phosphorus (NS); but diminution of this element caused a decrease in the moisture content of the shelled peas (A = B = C = D > F > G).

POTASSIUM AND BORON EXPERIMENTS

The 70 glazed culture jars were arranged as 10 replications of the 7 treatments A–G, Table 1. Seed sown, 7 July 1941; germinated by 12 July; seedlings singled, 21 July; and cultures harvested, 15 Sept. 1941. Each culture received 14·8 l. of its appropriate solution in lots of 400 c.c. about four times weekly.

During the period 7–21 Aug., flowering commenced for each treatment. A–F gave similar

TABLE 3. *Summaries of results for phosphorus experiment with peas. Weights in g.*

Description of data		Treatment mean for A	B	C	D	E	F	G	Mean of all results	S.E.
Whole tops, FW	SSS	36·92	42·15	41·34	44·43	47·21	42·45	6·78	37·33	2·838
		E>A>G; A=B=C=D=F>G; B=C=D=E=F								
Roots, FW	SSS	7·01	11·10	10·00	10·08	11·58	15·38	1·29	9·49	1·149
		F>B=C=D=E>G; B=E>A>G; A=C=D								
Whole plants, FW	SSS	43·93	53·25	51·34	54·50	58·79	57·83	8·07	46·82	3·500
		D=E=F>A>G; A=B=C>G; B=C=D=E=F								
Shelled peas, FW	SSS	8·29	10·34	11·03	13·44	14·01	14·73	1·82	10·52	0·772
		D=E=F>A=B=C>G								
Whole tops, DW	SSS	7·52	8·26	8·24	8·74	10·19	9·71	1·54	7·74	0·486
		E>A=B=C>G; D=E=F>G; E=F>A=C; A=B=C=D; B=C=D=F								
Roots, DW	SSS	0·97	1·64	1·71	1·64	1·60	1·83	0·22	1·37	0·272
		F>A=G; B=C=D=E=F>G; A=B=C=D=E								
Whole plants, DW	SSS	8·49	9·90	9·95	10·38	11·79	11·55	1·76	9·12	0·632
		E=F>A>G; A=B=C=D>G; B=C=D=E=F								
Shelled peas, DW	SSS	1·99	2·47	2·59	3·09	3·56	3·96	0·53	2·60	0·199
		F>D>A=B>G; E=F>A=B=C>G; D=E>A=B								
Whole top/root, FW	NS	5·57	4·91	8·11	5·06	4·70	2·91	5·78	5·29	1·600
Whole top/root, DW	NS	9·31	7·70	8·11	6·34	6·94	5·71	7·20	7·33	1·227
Whole tops, % moisture	NS	79·39	80·41	78·77	79·47	78·36	77·09	76·98	78·64	1·043
Roots, % moisture	NS	86·00	85·90	79·22	83·05	85·33	88·07	81·95	84·22	2·606
Whole plants, % moisture	NS	80·52	81·52	79·24	80·21	79·81	79·98	78·02	79·90	1·015
Shelled peas, % moisture	SSS	76·28	76·23	76·63	76·76	74·55	73·13	70·80	74·91	0·692
		A=B=C=D>F>G; C=D>E=F>G; A=B=E								

TABLE 4. *Summaries of results for potassium and boron experiments with peas. Weights in g.*

Description of data		Treatment mean for A	B	C	D	E	F	G	Mean of all results	S.E.
Whole tops, FW	SSS	43·20	36·21	42·90	40·73	38·64	32·90	8·47	34·72	1·625
		A=C>B=F>G; A=C=D=E>F; B=D=E								
Roots, FW	SSS	7·66	6·07	9·57	7·84	9·39	8·66	1·43	7·23	0·609
		C>A=B>G; C=E=F>B>G; C=D=E=F>G; A=D=E=F; A=B=D								
Whole plants, FW	SSS	50·86	42·29	52·47	48·57	48·03	41·56	9·90	41·95	1·914
		A=C=D=E>B=F>G								
Shelled peas, FW	SSS	6·17	5·23	6·83	7·30	7·76	5·52	0·32	5·29	0·637
		E>B=F; D=E>B; A=C=D=E>G; A=B=C=F>G; A=C=D=F>G								
Whole tops, DW	SSS	6·35	5·39	6·67	6·19	5·84	4·68	1·50	5·23	0·252
		A=C=D>B=F>G; C>E>F>G; A=D=E; B=E								
Roots, DW	SSS	0·70	0·59	0·82	0·64	0·71	0·61	0·13	0·60	0·052
		C>B=D=F>G; A=C=E; A=B=D=E=F>G								
Whole plants, DW	SSS	7·05	5·98	7·49	6·83	6·55	5·28	1·63	5·83	0·272
		A=C=D>B=F>G; C>E>F; A=D=E>F; B=E								
Shelled peas, DW	SSS	1·10	0·93	1·23	1·29	1·41	0·86	0·04	0·93	0·134
		E>B=F>G; A=C=D=E>G; D=E>F; A=B=C=D; A=B=C=F								
Whole top/root, FW	SS	5·67	6·10	5·02	5·51	4·33	3·87	6·62	5·30	0·510
		A=B=D=G>F; B=G>E=F; A=B=C=D; G>C=E=F; A=C=D=E								
Whole top/root, DW	NS	9·43	9·34	8·75	10·31	8·65	7·97	13·05	9·64	1·142
Whole tops, % moisture	SS	85·16	85·11	84·51	84·79	84·94	85·80	78·24	84·08	1·323
		A=B=C=D=E=F>G								
Roots, % moisture	SS	90·91	90·25	91·11	91·83	92·37	93·04	89·13	91·23	0·681
		F>A=B=G; E=F>B=G; D=E=F>G; C=D=E=F; A=C=D=E; A=B=C=D; A=B=C=G								
Whole plants, % moisture	SS	86·04	85·85	85·74	85·94	86·39	87·30	80·00	85·32	1·261
		A=B=C=D=E=F>G								
Shelled peas, % moisture	SSS	82·38	82·62	82·15	82·61	82·33	84·86	88·43	83·79	0·796
		G>F>A=C=E; G>D=F; A=C=D=E								

cultures. With G, small plants, about 9 in. high, were obtained; and the leaves had developed marginal scorch progressively up the stem for about 4 in., then extensive scorch, and then had died. The result was a sturdy looking, bushy plant on top of a stem, instead of the normal rambling plant.

Treatments A–F all showed branched main stems at harvest, though the branches with F were of recent growth. The numbers of plants with pods on were: A, 10; B, 6; C, 10; D, 10; E, 10; F, 10; and G, 10. The average numbers of pods per culture were: A, 9·4; B, 3·5; C, 8·4; D, 7·6; E, 8·3; F, 8·3; and G, 4·1. The few pods with B, absence of boron, were remarkably plump, so that a good yield of peas was obtained even though only six of the ten cultures had pods. The numbers of cultures possessing root nodules were: A, 8; B, 10; C, 9; D, 8; E, 10; F, 7; and G, 8. Some plants of all treatments but G were suffering from slight attacks of mildew. With F, the lower foliage for about 6 in. up the old main branch had died of scorch; with extreme deficiency of potassium, G, the foliage on the original stems had mostly died of scorch working progressively up the stem, and a very short, bushy clump of dark green foliage, already showing scorch of the lower leaves, had commenced to grow from the base of the stem of all the cultures.

In Table 4, the summaries of results for the 14 subtables are given. Solution B (absence of boron, but otherwise identical with solution C) did not result in great depressions in yield, and it must be assumed that the large seed normally contains an appreciable amount of boron, or that the pea plant needs little. For fresh or dried shelled peas the yields were a maximum, and constant, from E–A, 5·61–44·88 p.p.m. of potassium (A = C = D = E); it may be assumed, therefore, that this is the optimum range for seed production, and that the lower portion of this range is naturally the most economic.

Extreme deficiency of potassium, G, 0·28 p.p.m., caused a diminution in moisture content of the whole tops and whole plants, while absence of boron had no effect in this regard (A = B = C = D = E = F > G); the reverse effect was noted for the moisture content of the shelled peas (G > F > A = C = E; G > D = F). Treatment B, absence of boron, was omitted from the comparisons of this subtable because some of the cultures gave no pods or peas, and therefore statistical treatment was difficult; the italicized figure given as the mean of the available data, however, showed this moisture content to be little different from the values for all other treatments except F and G.

I thank Mr J. F. Leonard for his help in these experiments.

REFERENCES

WOODMAN, R. M. (1940). The nutrition of lettuces grown as sand cultures under glass. *Ann. appl. Biol.* **27**, 5–16.

WOODMAN, R. M. (1942). The nutrition of spinach. *Ann. appl. Biol.* **29**, 97–102.

(*Received* 7 *May* 1942)

The nutrition of vegetables in sand

BY R. M. WOODMAN, *Horticultural Research Station, School of Agriculture, Cambridge University*

(With 21 Text-figures)

The yield data available from the author's series of investigations on vegetable nutrition in sand have been plotted against the concentrations of the elements concerned (nitrogen, phosphorus, and potassium), and the curves so obtained have been discussed. Within the ranges of concentrations employed, it is observed that too much available nitrogen or, especially, available phosphorus, causes depressions in yields with the tops and/or roots of certain vegetables, turnip roots being particularly susceptible to too much available phosphorus; no case of over-employment of available potassium, however, is noted. The turnip is recommended as a type plant for indicating the state of soil fertility as regards available (nitric) nitrogen.

The present paper contains a digest of the yield data from the author's work on the nutrition of vegetables in sand.

Figs. 1–21 were obtained by plotting the treatment means, in g., of the yields (fresh and dry weights of tops, roots, and whole plants for each crop, and fresh and dried weights of shelled peas) against the concentrations in p.p.m. of the element under experiment. The root of a 'root crop' is taken to be the edible portion plus associated fibre root, as in

horticultural practice. The curves for potassium with the two varieties of lettuce are omitted, as it was later found that the waxed plant-pots used were unsuitable for potassium experiments (Woodman, 1941*a*).

Throughout the experiments seventy glazed culture jars were available. This limited number necessitated separate experiments at different seasons for any particular crop under investigation, instead of the single comprehensive experiment mentioned previously as probably being desirable (Woodman, 1939*a*). Such a method of experimentation resulted in certain disadvantages: the amount of growth was naturally different in the separate experiments for any given crop, because of variation in such factors as length of day. Second- and third-order interactions had also to be excluded from consideration.

But the method also had definite advantages: although the number of pots was insufficient for a comprehensive experiment even at three levels of nitrogen, phosphorus, and potassium, it was sufficient for ten replications of seven levels with any one of them taken singly. The concentrations, therefore, could be stepped down gradually, and the ranges of concentrations where yields showed a decided alteration, and where deficiency symptoms began, ascertained with some degree of accuracy. The data for yields at about five or more levels were also necessary to obtain reasonably correct shapes for curves such as those shown in Figs. 1–21.

Moreover, this method led to knowledge concerning the different requirements of individual crops for any elements; thus, for example, the varying requirements of the turnip and the radish roots as regards nitrogen (Fig. 2) would probably have been wholly obscured by taking three levels only of nitrogen (including nil, of course) in a multiple-factor experiment. It seems, therefore, that the ideal method of experiment with cultures would be first to vary each element separately in several steps to obtain detailed information about the element under consideration, and then to make a comprehensive experiment, with, e.g., three selected levels of nitrogen, potassium, and phosphorus, in order to obtain interactions.

Explanation of, and keys to, the figures, with references to the original data

The abscissae are on the same scale for Figs. 1–21, and represent p.p.m. of the variable element. The ordinates represent the yields (treatment means in g.); these cannot be conveniently put on the same scale because of the wide divergencies in yield for different vegetables, and so more than one scale has been employed in most of the figures.

Certain of the curves have been smoothed to some extent; but the shapes were chosen to conform with the comparisons of statistical significance tabulated in the second line of each yield subtable of the Tables of the summaries of results given in the papers referred to below. Thus, where a maximum occurs on a curve, this corresponds to a significant difference in yield, or to a decided bias that has been usually proved by further statistical examination of the results to possess some degree of significance. For one curve only, that for the dry weights of pea roots, were the results not significant as a whole (Fig. 5, IV).

The curves for *nitrogen nutrition* are shown in Figs. 1–7. For each of Figs. 1–6, curve I is for Messrs Sutton's 'Harbinger' spring cabbage (data, Woodman, 1941*b*); curve II for Messrs Watkins & Simpson's 'Purple Top' turnip (Woodman, 1941*a*); curve III for Messrs Watkins & Simpson's 'May King' lettuce (Woodman, 1939*b*); curve IV for Messrs Charles Sharpe's 'Lincoln' pea (Woodman, 1944); curve V for Messrs Unwin's 'Unwin's Reliance' onion (Woodman, 1943*b*); curve VI for Messrs Watkins & Simpson's 'Primo' carrot (Woodman, 1943*a*); curve VII for Messrs Clucas's 'Giant-leaved' spinach (Woodman, 1942); curve VIII for Messrs Watkins & Simpson's 'Turnip, Red, Extra Early, Short Top Forcing' radish (Woodman, 1943*c*); and curve IX for 'Cheshunt Early Giant' lettuce (Woodman, 1940). Curves I and II, Fig. 7, are for the fresh and dried shelled peas respectively (Woodman, 1944).

Curves for *phosphorus nutrition* are given in Figs. 8–14; the references are as above, except for 'May King' lettuce. For Figs. 8–13, curve I is for the cabbage, II for the turnip, III for 'May King' lettuce (Woodman, 1939*a*), IV for the pea, V for the carrot, VI for the spinach, VII for the radish, and VIII for 'Cheshunt Early Giant' lettuce. Curves I and II, Fig. 14, are for fresh and dried shelled peas respectively.

Figs. 15–21 show the yield curves for *potassium nutrition*, references again being as above. In Figs. 15–20, curve I is for the cabbage, II for the turnip, III for the pea, IV for the carrot, V for the spinach, and VI for the radish. Curves I and II, Fig. 21, are for the fresh and dried shelled peas respectively.

Nitrogen nutrition

The curves for the fresh weights of tops all show an initial steep portion (Fig. 1). That cabbages (curve I), turnips (II), onions (V), and radishes (VIII), require nitrogen for good growth of tops is shown by the fact that maximum yields are not reached even at 65·92 p.p.m. of nitrogen. These curves may therefore be said to be 'normal' as regards response to nitrogen, as an increase in yield follows an increase in fertilizer, though whether it would be economical to apply nitrogen at concentrations corresponding to the flat, upper portions of these curves is a moot point. With curves III and IX for the two lettuces, the range of concentrations employed, 0–32·96 p.p.m.,

was not wide enough to indicate maximum growth. The last portions of the curves for pea haulms (IV), spinach foliage (VII), and carrot tops (VI), indicated that there was either no response to nitrogen above certain concentrations, or else a tendency to a diminution in yield; fertilizer applied in such amounts would obviously be wasted.

Cabbage tops and spinach foliage are the marketable portions of these vegetables. Nitrogen concentrations above 32·96 p.p.m. result in unmarketable cabbages because of scorch (Woodman, 1941*b*); curve I begins to flatten out at about 40 p.p.m., so that increase of fertilizer beyond 30–40 p.p.m. is possibly not warranted from the points of view of both marketability (quality) and yield. Spinach tops (VII) do not respond to more than about 30 p.p.m. of nitrogen; as, however, extra nitric nitrogen tends to a better colour and to a lessened tendency to flower-stem formation and elongation, nitrogen in excess of this amount is probably desirable (Knott, 1940; Woodman, 1942).

Turnip roots (II, Fig. 2) also respond 'normally' and well to nitrogen. On the other hand, the carrot (VI) requires little, about 10 p.p.m., for optimum growth; the radish (VIII) requires about 25 p.p.m., and the onion (V) a somewhat similar amount. With these three roots, greater concentrations produce either no response, or, as with the onion, an actual diminution in yield. The other roots are commercially unimportant, but the maximum for spinach (VII) is significant, though peculiar.

Curves for the fresh weights of the whole plants, Fig. 3, are more or less compounds of those in Figs. 1 and 2, the relative proportions of the tops and roots for any given vegetable determining the shape of the curve for the whole plant.

Curve II for the turnip emphasizes the fact that this would make nearly an ideal 'type' vegetable for nitrogen. Thus both roots and tops respond well over a wide range of nitrogen, and the responses are such as can be easily detected by the balance or by the eye; the crop is relatively quick-growing (about 10 weeks normally in the greenhouse), and begins to show responses to nitrogen early, so that it would be unnecessary to grow it to maturity. The heads of the two lettuces and spring cabbage (III, IX, and I, Fig. 1) could also be used as indicators for nitrogen up to 30 p.p.m., as the gradients of the curves are steep to that point, and the yields fairly large.

It is not necessary to comment in detail on Figs. 4–6. The turnip shows clear responses over the whole range of concentrations for tops, roots, and whole plants, the gradients of the curves for this vegetable being such as to enable that easy differentiation of response to an element desirable in a type plant.

Fig. 7 demonstrates the response of fresh shelled peas (I) and dry matter of the shelled peas (II) to nitrogen. There are definite maxima here, and it is obvious that the optimum yields lie approximately between 7·5 and 20 p.p.m., a somewhat narrower range than could be determined from a mere examination of the yields, or from the statistical treatment (Woodman, 1944, Table 1). The gradients of the two curves indicate that it would be much more serious to reduce the lower limit of this range from 7·5 to, say, 4–5 p.p.m., than it would be to increase the upper limit from 20 to 25 or even to 30 p.p.m.; that is, there is more danger of loss of yield from lack of nitrogen than from an excess of it. This finding may be noted for all the curves in Figs. 1–7 for nitrogen nutrition, and is correlated with the fact that the initial portions of all of them have the steepest gradients.

As all nitrates are soluble, they should be present in the soil solution [and, in fact, the concentration of nitrate in the soil does vary inversely as the moisture content (Russell, 1937)]. This indicates that, for nitric nitrogen, sand cultures are equivalent to actual practice with soils: the added culture solution should be, at least in this respect, strictly comparable to the soil solution.

Thus, for example, the range of nitrogen for optimum yield of shelled peas, about 7·5–20 p.p.m., is approximately equivalent to a range of 33–89 (say 30–90) p.p.m. of nitrogen as $(NO_3)'$. For optimum yield of shelled peas, therefore, the concentration of nitrate in the soil solution should lie between the limits 30–90 p.p.m. as $(NO_3)'$. The amount of soluble nitrogen to be added to the pea crop thus depends on the original nitrate content of the soil solution, which in turn is a variable dependent on several factors, such as amount of soil moisture, the action of the plant, the action of soil organisms, and the time of the year.

Phosphorus nutrition

Curves for the fresh tops of the lettuces, 'May King' and 'Cheshunt Early Giant', and for carrots (Fig. 8, III, VIII and V, respectively), are 'normal' within the limits of the ranges of concentration used (the upper limit for the carrot is low). The commercial portion of the lettuce thus responds to phosphorus; but cabbage heads (I) do not, beyond a certain concentration, about 6 p.p.m. Indeed, the tops of all the vegetables examined, except the three specially mentioned above, give maximum yields at low values for phosphorus, as may easily be seen from the curves. In this connexion, however, it should be borne in mind that all the phosphorus applied to the sand cultures was soluble and available (disodium hydrogen phosphate), with little possibility of interactions of any kind with the pure sand employed (99·8% SiO_2), whereas in the soil solution the available phosphorus is low, and usually but a small proportion of the total of that element in the whole soil.

Lettuces again show 'normal' behaviour for the

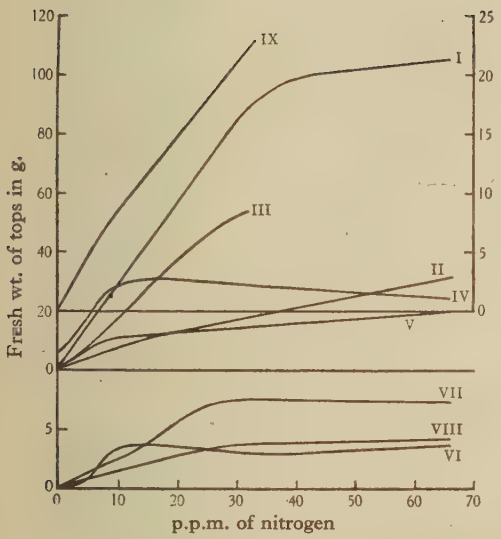


Fig. 1.

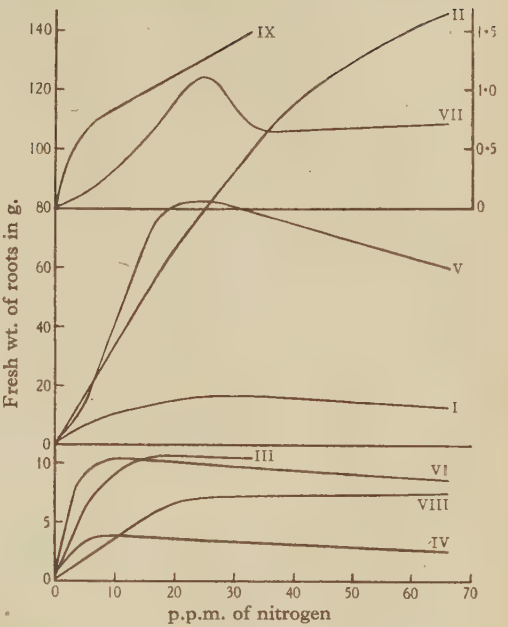


Fig. 2.

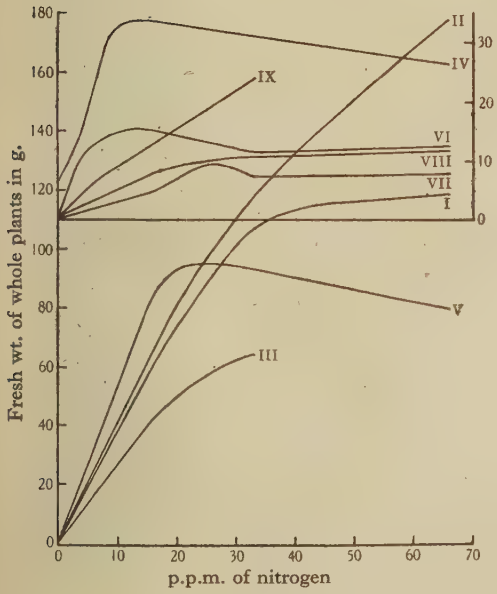


Fig. 3.

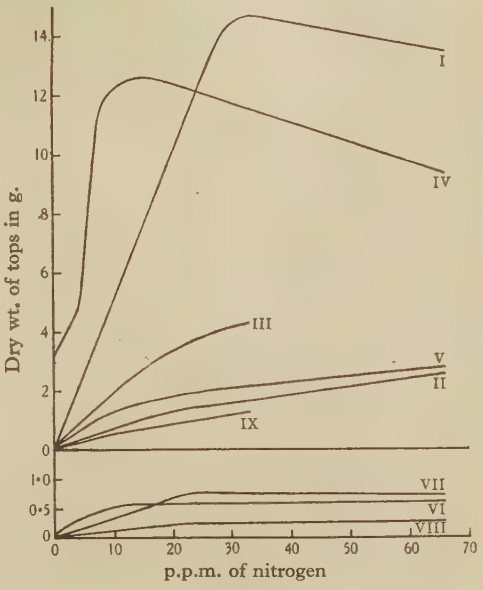


Fig. 4.

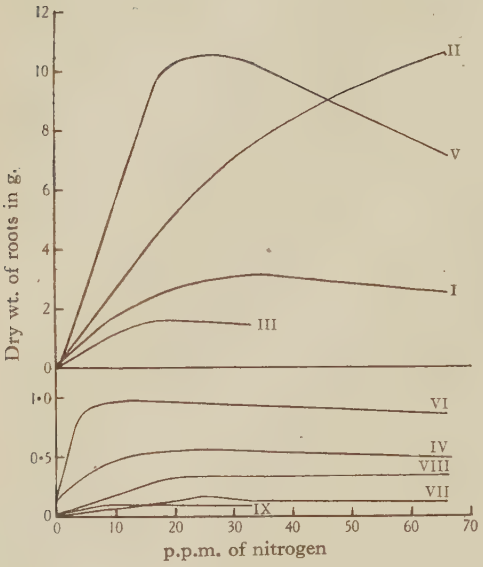


Fig. 5.

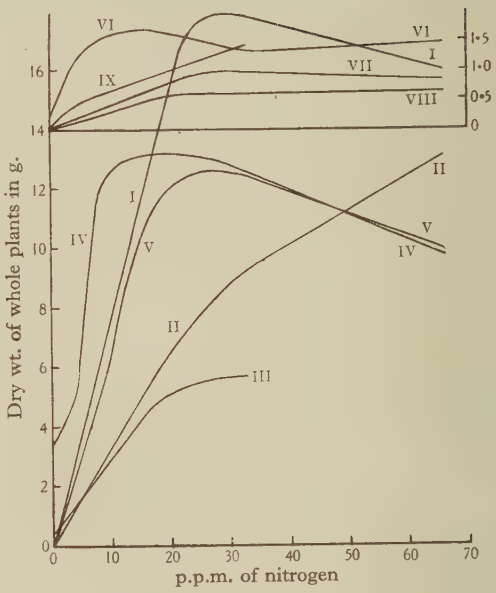


Fig. 6.

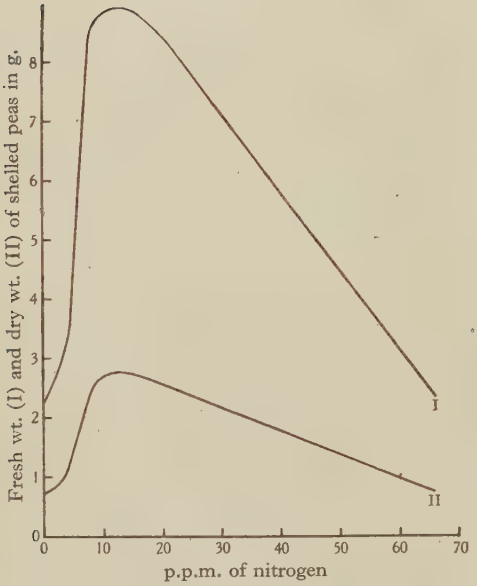


Fig. 7.

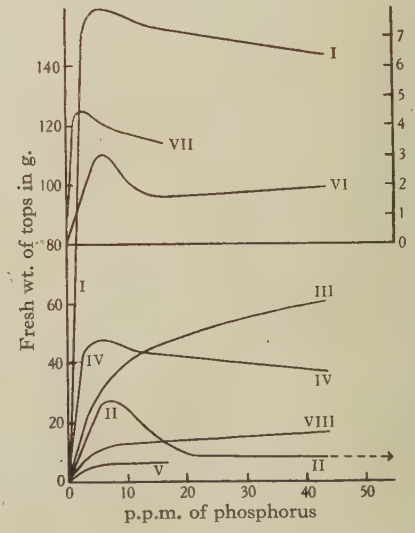


Fig. 8.

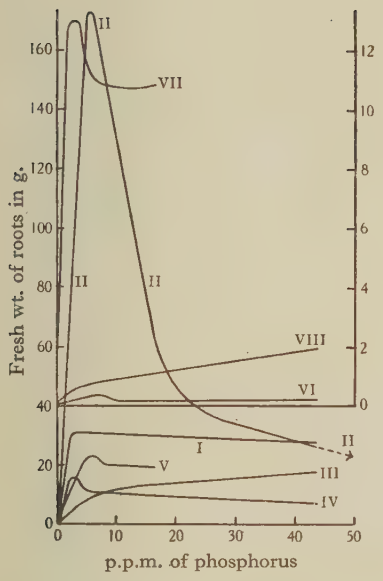


Fig. 9.

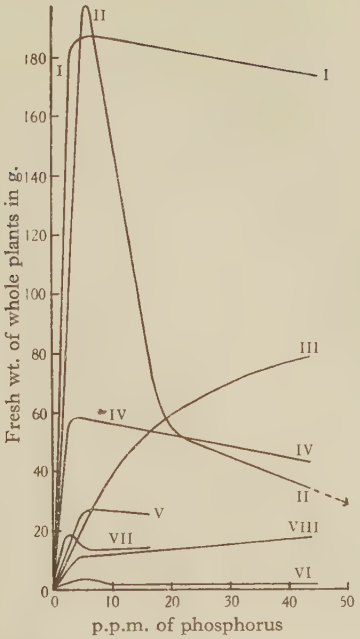


Fig. 10.

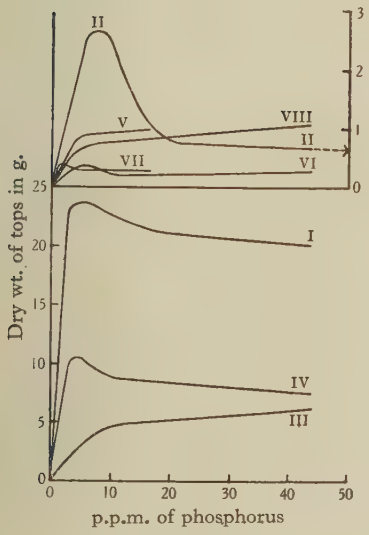


Fig. 11.

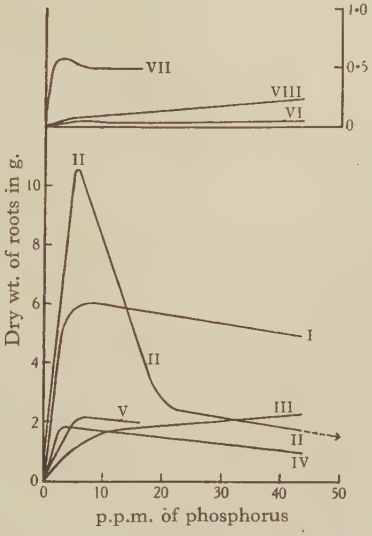


Fig. 12.

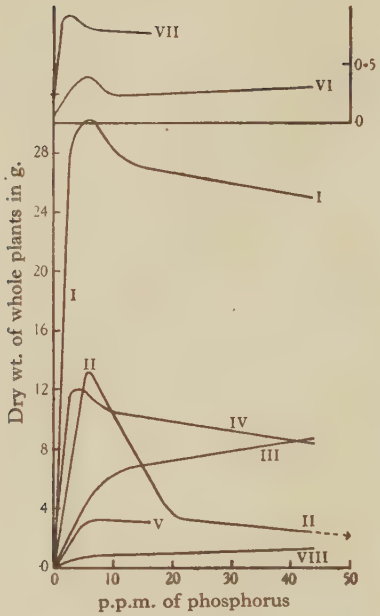


Fig. 13.

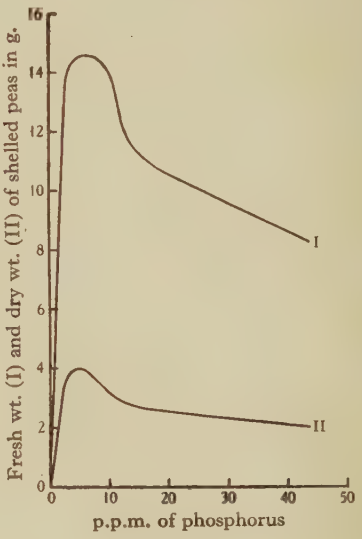


Fig. 14.

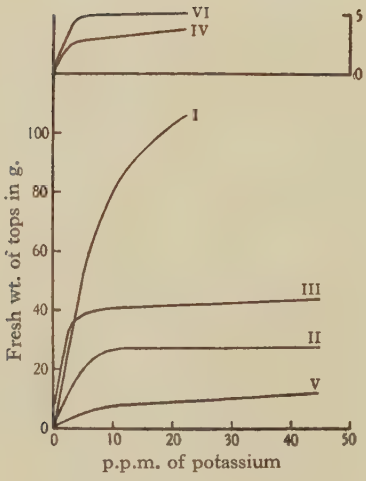


Fig. 15.

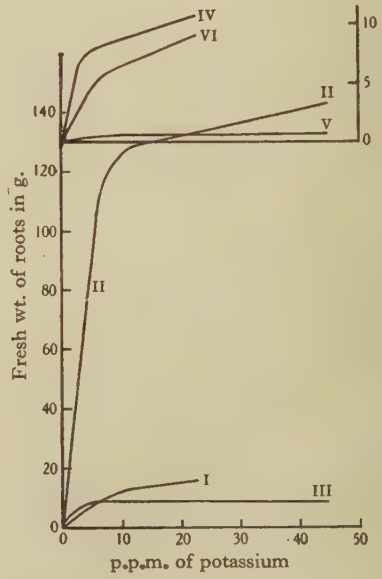


Fig. 16.

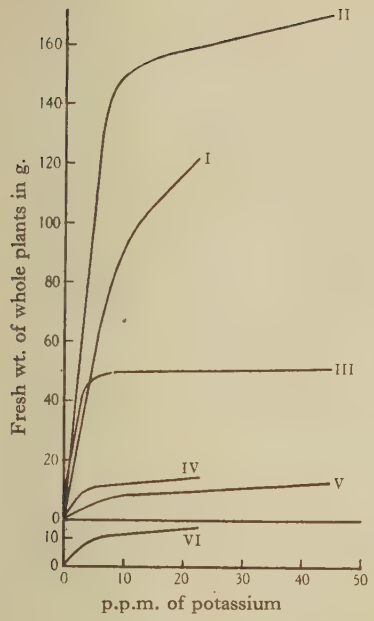


Fig. 17.

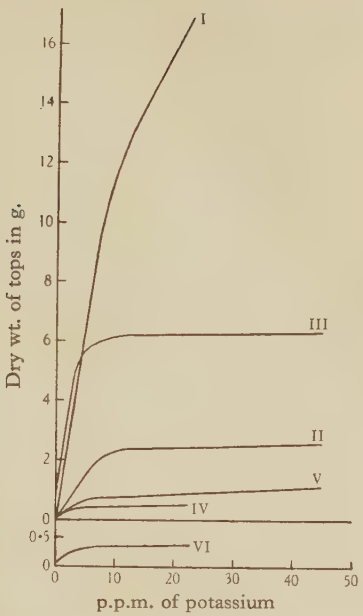


Fig. 18.

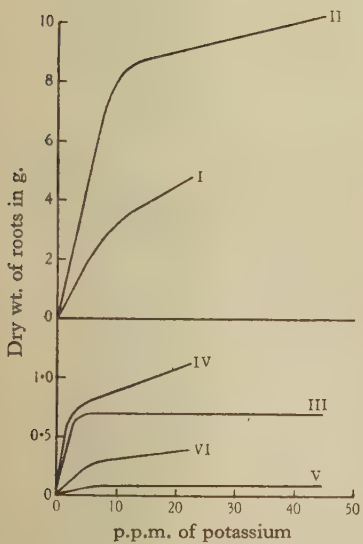


Fig. 19.

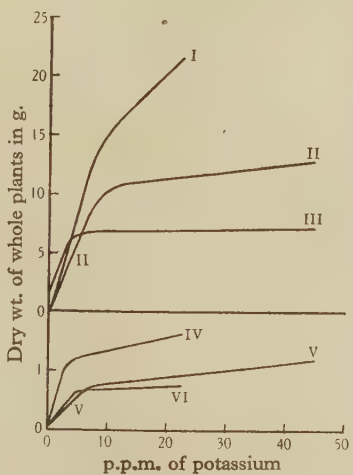


Fig. 20.

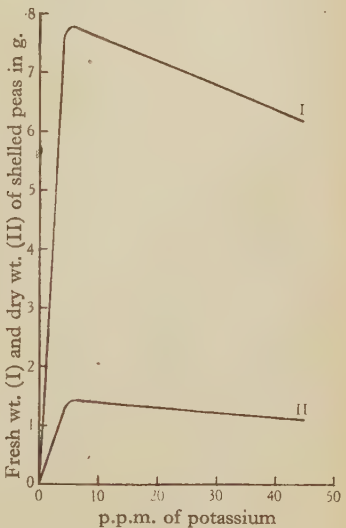


Fig. 21.

fresh roots, Fig. 9, III and VIII, while the other crops give maximum yields at low values of soluble phosphorus. The most striking curve in this respect is that for the turnip root (II). Here increases in available phosphorus from 0 to 5–6 p.p.m. cause corresponding and almost directly proportional increases in yield from practically zero to about 173 g.; further increase in phosphorus then causes such a diminution in yield that over the range 6–17 p.p.m. the reduction is from 173 to 58 g. The narrow range 5–6 p.p.m. of available phosphorus is thus the optimum one, and the practical aim should be to maintain the concentration of phosphorus in the soil solution at this level. The difference in behaviour between lettuces and certain other vegetables as regards response to available phosphorus has been noted previously by the author (Woodman, 1943*a*).

Figs. 10–13 show curves somewhat similar to Figs. 8 and 9. Fig. 14 demonstrates that there are also maximum yields for fresh and dried peas at a low range of available phosphorus.

Potassium nutrition

From Figs. 15–20 it is clear that there is no maximum on any curve for any of the six yields for all the vegetables concerned, every curve demonstrating 'normal' response to available potassium up to the limits of the concentrations employed. The responses are greatest on the initial, steepest portions of the curves, which then tend to flatten out and sometimes become practically parallel to the abscissae, as with fresh turnip tops, Fig. 15, II, and fresh pea and spinach roots, Fig. 16, III and V. This raises the question as to whether or not it would be economical to apply potassium in excess of a certain amount for each vegetable; but whatever the answer, the facts remain that up to the upper limit of concentrations used, 44·88 p.p.m. of potassium, there was no adverse effect on yield and certainly none on the health of the vegetables. Employment of more than 6 p.p.m., however, did cause some reduction in the yield of shelled peas, fresh or dried, Fig. 21.

With potassium, as with phosphorus, it is undesirable to press too far any analogy between sand cultures and actual practice with soils, as much of this element in soils is not available in the soil solution. The most that can be stated is, that to obtain good yields of the vegetables grown, the soil solution should contain concentrations of potassium at least equal to those where the curves begin to flatten out.

REFERENCES

Knott, J. E. (1940). Effect of nitrate of soda on the response of spinach to length of day. *Plant Physiol.* **15**, 146–8.

Russell, E. J. (1937). *Soil Conditions and Plant Growth*, 7th ed. p. 531.

Woodman, R. M. (1939*a*). Phosphate deficiency and yield tests on sand cultures of May King lettuce. *J. agric. Sci.* **29**, 229–48.

—— (1939*b*). The effects of variation in the nitrogen supply on lettuce (var. May King) in sand culture. *Ann. Bot., Lond.*, N.S. **3**, 649–56.

—— (1940). The nutrition of lettuces grown as sand cultures under glass. *Ann. appl. Biol.* **27**, 5–16.

Woodman, R. M. (1941*a*). The nutrition of turnips. *Ann. appl. Biol.* **28**, 1–7.

—— (1941*b*). The nutrition of the spring cabbage. *Ann. appl. Biol.* **28**, 181–8.

—— (1942). The nutrition of spinach. *Ann. appl. Biol.* **29**, 97–102.

—— (1943*a*). The nutrition of the carrot. *Ann. appl. Biol.* **30**, 1–7.

—— (1943*b*). Nitrogen nutrition of the onion. *Ann. appl. Biol.* **30**, 116–17.

—— (1943*c*). The nutrition of the radish. *Ann. appl. Biol.* **30**, 319–22.

—— (1944). The nutrition of the pea. *Ann. appl. Biol.* **31**, 19–22.

(*Received* 5 *November* 1942)

Notes on basal rot of narcissus

III. Eradication of the disease from narcissus stocks by repeated use of formalin in the hot-water bath

By Lilian E. Hawker, *Department of Mycology and Plant Pathology, Imperial College of Science and Technology, London*

(With Plate 2)

Stocks of narcissi infected with *Fusarium bulbigenum* were divided into two parts, of which one was given the standard hot-water treatment and the other was hot-water treated with the addition of 0·5 % formalin to the water. Some stocks were lifted the following season and the treatment was repeated. With most stocks a single application of formalin was sufficient to reduce the amount of disease to negligible proportions, but with one heavily infected stock a second treatment was necessary.

Gregory (1932) suggested that damage to narcissus bulbs from basal rot (caused by *Fusarium bulbigenum* Cke. & Mass.) following hot-water treatment against eelworm could be reduced by the addition of formalin to the water. Experiments on the control of the disease by this method have since been described by Hawker (1935, 1940, 1943) and by other investigators (Moore, 1939). This treatment was adopted at a large bulb farm where losses from basal rot, particularly among valuable white trumpet daffodils, were severe. Bulbs were usually lifted every third year and were hot-water treated with formalin during the following storage season. After a number of such treatments all stocks were practically free from basal rot. It was not clear whether this was due to the routine use of formalin or to a succession of dry seasons which might have been unfavourable to the fungus. Experiments with seven stocks naturally or artificially contaminated with *F. bulbigenum* were therefore laid down by the writer at the Imperial College of Science, Biological Field Station at Slough. Half of each stock was given the normal hot-water treatment, while the other half was hot-water treated with the addition of 0·5 % formalin to the water. The bulbs were then planted in ground where narcissi had not previously been grown. Two stocks were treated in 1939 and lifted in 1940. The treatments were repeated and the bulbs were replanted in fresh ground together with five other treated stocks. All were lifted in 1943. It was originally intended to treat and replant all the stocks in 1943, but the results were sufficiently conclusive to render continuation of the experiment unnecessary.

Table 1 shows the effect of formalin on two naturally infected stocks of var. Bicolor Victoria. Stock A, which was originally heavily infected, was lifted at the end of the first growing season after treatment. The hot-water treated half was seriously reduced by the disease while the formalin-treated lot, although still showing an appreciable amount of basal rot, had increased in weight. The diseased bulbs were discarded and the survivors were given the same treatments as before and were left down for three growing seasons. The second treatment with formalin reduced the amount of disease almost to zero, while the control batch remained heavily infected. Stock B, which had less initial infection, was practically freed from the disease by a single formalin treatment. With both stocks formalin increased the gain in weight and the number of flowers and reduced the numbers of poor and missing plants and of bulbs rotting with *Fusarium* when lifted or in subsequent storage.

Five other stocks showed a negligible amount of basal rot after only one treatment with formalin, while the disease progressed in the controls hot-water treated without any fungicide. The increase in weight during three growing seasons after treatment was greater, flowers were more numerous and there were fewer poor and missing plants and fewer bulbs rotting from *Fusarium* either when lifted or during storage in the formalin-treated lots (Table 2). Pl. 2, figs. 1 and 2, shows twelve rows of Bicolor Victoria (stock C) in the third spring after hot-water treatment with and without formalin respectively. The contrast between the two lots with respect to number of flowers, proportion of fully opened blooms and quality and number of surviving plants is clear.

These results indicate that the routine use of 0·5 % formalin in the hot-water bath will eradicate basal rot from even severely infected stocks. At the bulb farm already mentioned a proprietary brand of formalin (designed to prevent polymerization) was used in sufficient quantity to give a formaldehyde content equal to that in 0·2 % commercial formalin.

A small-scale experiment at Slough indicated that this proprietary preparation was as good as commercial formalin of equivalent strength but that, with heavily infected stocks, the higher dose gave better results. The lower dose (0·2 % formalin) gave satisfactory results with lightly infected stocks.

TABLE 1. *Effect of two treatments with formalin*

Stock	Victoria (stock A)		Victoria (stock B)	
Treatment	H.W.T.	H.W.T. + F.	H.W.T.	H.W.T. + F.
No. planted 1939	400	400	240	240
% increase in wt. 1939–40	−56·0	+93·5	+ 8·8	+61·4
Total flowers 1940	383	482	452	472
Plants missing 1940	6	2	5	0
Poor plants 1940	26	0	14	1
Bulbs rotting from *Fusarium* when lifted 1940	170	23	80	0
Bulbs rotting from *Fusarium* in storage 1940	38	15	40	7
No. planted 1940	135	569	400*	540†
% increase in wt. 1940–3	+10·9	+249	+29·8	+196·0
Total flowers: 1941	142	405	305	449
1942	291	927	553	776
1943	177	866	563	946
Plants missing: 1941	3	1	3	0
1942	15	4	20	3
1943	26	16	59	6
Poor plants: 1941	13	7	12	4
1942	16	14	30	5
1943	21	16	31	6
Bulbs rotting from *Fusarium* when lifted 1943	50	4	44	4
Bulbs rotting from *Fusarium* in storage 1943	9	1	14	2

* Including 240 offsets and small bulbs.

† Including 300 offsets and small bulbs.

H.W.T. = hot-water treatment.

H.W.T. + F. = hot-water treatment + 0·5 % formalin.

TABLE 2. *Effect of a single treatment with formalin*

Stock	Victoria (stock C)		Spring Glory		Henry Irving		Glory of Leiden		Grandis	
Treatment	H.W.T.	H.W.T. + F.	H.W.T.	H.W.T. + F.	H.W.T.	H.W.T. + F.	H.W.T.	H.W.T. + F.	H.W.T.	H.W.T. + F.
No. of bulbs treated	165	165	150	150	330	330	280	280	40	40
Total flowers: 1941	260	276	149	121	269	296	158	196	9	10
1942	224	447	199	224	281	366	333	412	43	73
1943	196	409	188	227	415	467	182	347	23	104
Plants missing: 1941	5	3	4	0	3	0	3	0	0	0
1942	48	7	6	3	29	0	9	2	7	0
1943	115	11	12	3	51	1	28	8	15	1
Poor plants: 1941	17	4	6	6	2	1	10	1	2	1
1942	47	5	13	5	11	4	11	8	5	0
1943	54	2	15	5	11	2	23	8	21	1
% increase in wt.	49·5	199·7	146	193·3	34·4	60·8	82·7	134·2	−25·4	224·7
Bulbs rotting from *Fusarium* when lifted 1943	40	3	8	0	32	0	15	1	18	1
Bulbs rotting from *Fusarium* in storage 1943	16	0	2	0	7	0	18	2	4	1

H.W.T. = hot-water treatment; H.W.T. + F. = hot-water treatment + 0·5 % formalin.

Fig. 1

Fig. 2

REFERENCES

GREGORY, P. H. (1932). The *Fusarium* bulb rot of narcissus. *Ann. appl. Biol.* **19**, 475–514.

HAWKER, L. E. (1935). Further experiments on the *Fusarium* bulb rot of narcissus. *Ann. appl. Biol.* **22**, 684–708.

—— (1940). Experiments on the control of basal rot of narcissus bulbs caused by *Fusarium bulbigenum* Cke & Mass., with notes on *Botrytis narcissicola* Kleb. *Ann. appl. Biol.* **27**, 205–17.

HAWKER, L. E. (1943). Notes on basal rot of narcissus. I. A comparison of various methods of using formalin in connexion with the hot-water treatment against eelworm. *Ann. appl. Biol.* **30**, 323.

MOORE, W. C. (1939). Diseases of bulbs. *Bull. Minist. Agric. Lond.* no. 117, pp. 176.

EXPLANATION OF PLATE 2

Fig. 1. Twelve rows of var. Bicolor Victoria (stock C) photographed in the third growing season after hot-water treatment with formalin. Note full stand and numerous flowers.

Fig. 2. Similar stock hot-water treated without formalin. Note poor stand, weak foliage and few flowers.

(*Received 7 October* 1943)

The relationships of some viruses causing necrotic diseases of the potato

BY F. C. BAWDEN AND F. M. L. SHEFFIELD
Rothamsted Experimental Station, Harpenden, Herts

(With Plate 3)

Potato virus *B*, and some other viruses with reactions in potato varieties different from any previously described, are strains of virus *X*. All produce intracellular inclusions which vary with different hosts and virus strains. Except with virus *B*, the inclusions are larger and more frequent in potato than in tobacco or tomato. All give systemic infection when inoculated to tobacco, tomato and potato varieties in which they are carried or cause mosaic symptoms; some give systemic infection when inoculated to varieties in which they cause top-necrosis, whereas others give only local lesions.

Potato virus *C* is a strain of virus *Y*: in tobacco and a few potato varieties both produce similar symptoms, but in those varieties in which *Y* causes leaf-drop streak, *C* causes top-necrosis. *C* causes systemic infection when inoculated to tobacco and to potato varieties in which it causes mosaic symptoms, but not when inoculated to potato varieties in which it causes top-necrosis. Virus *C* was not transmitted by *M. persicae*. Viruses *C* and *Y* produce a few small intracellular inclusions in potato and tobacco.

Virus *A* is not related to *Y* or *X*: no inclusions were found in plants infected with *A* alone.

Top-necrosis, or acronecrosis (Quanjer, 1931), is a lethal disease of the potato rarely seen in the field but often met in experimental work involving the grafting of one potato variety to another. It is caused by a number of viruses and virus strains, and Bawden (1936) showed that some of these could conveniently be identified by using the varieties Arran Victory, President, Epicure and Up-to-Date as differential hosts. The results of transmission tests from carrier plants of the varieties Up-to-Date and Di Vernon to these differential hosts were most simply explained by assuming infections with mixtures of viruses, some of the constituents of which differed from any previously described. The letters *B* and *C* were used for the specific 'new' viruses postulated in Up-to-Date and Di Vernon respectively. At that time there was no method of separating virus *B* or *C* from virus *X*, which accompanied them in all the plants tested, so that no detailed studies of their properties could be made. Virus *B* resembled virus *X* in some of its properties, but it was believed to be a distinct virus rather than a strain, because *Datura stramonium* infected with *X* still seemed susceptible to infection with *B*.

Increased knowledge of viruses and virus diseases has shown that symptomatology affords no sure basis for the grouping of viruses, but the method of differential hosts was widely used for the identification of potato viruses, and the existence of viruses *B* and *C* was confirmed in various parts of the world (Dykstra, 1935, 1939; Scott, 1938; Cockerham, 1939*a*, *b*, 1941; Dennis, 1939; Bald & Pugsley, 1941;

Clinch, 1942). Virus *C* was apparently accepted by all workers as unrelated to any other potato virus, but there was difference of opinion as to the relations between viruses *B* and *X*. Dykstra, Dennis, Cockerham, and Scott, regarded them as separate viruses, whereas Bald & Pugsley, and Clinch, regarded them as related strains.

In the course of testing potatoes for freedom from virus diseases, Scott (1938) and Cockerham (1939*b*) found individuals of Duke of York and Edgecote Purple which appeared to be infected with viruses *B* and *C*, respectively, free from contaminating viruses. This material offered an opportunity to test some of the properties of the viruses and to determine their relationships with other viruses.

Material and methods

Much of this work was made possible by the generosity of Mr R. J. Scott, who kindly supplied us with potato tubers infected with viruses *B* and *C*. He also supplied a further virus suspected to be a strain of virus *X*, although its reactions on potatoes differed strikingly from those previously described, especially in that it did not cause top-necrosis in vars. Epicure and King Edward. Of the other viruses studied, some were supplied by Dr R. N. Salaman and others were found in naturally infected plants in the field.

Cytological studies were made on various potato varieties, tobacco (var. White Burley) and tomato (var. Kondine Red) infected with the viruses and virus strains. Fresh material, either hair cells, epidermal strippings or hand sections, was mainly used, but infected tobacco and potato leaves were also fixed and sectioned by the paraffin method. Zenker's solution following rapid immersion in Carnoy's solution was the most suitable fixative for both hosts, and Feulgen's reagent with a suitable counterstain and Heidenhain's haematoxylin were the strains most used.

Viruses *X* and *B*

In differentiating between potato viruses *X* and *B* (Bawden, 1936) two main features were used. First, virus *X* but not *B* became systemic when inoculated to Arran Victory and President, so that from the mixture of the two viruses a culture of *X* alone was obtained by needle-inoculation to these varieties. Secondly, potato virus *X* caused top-necrosis in Epicure but not in Arran Victory or President, whereas the mixture of the two viruses caused top-necrosis in all three varieties. At that time it could not be determined what effect virus *B* alone had on these varieties.

Inoculations from the Duke of York potato, supplied by Mr Scott as infected with virus *B* alone, to tobacco, *D. stramonium*, and tomato, produced reactions similar to those described by Salaman (1938) for the strain of virus *X* he called X^L. Tobacco and *D. stramonium* showed diffused chlorotic local lesions and later developed a systemic, yellow interveinal mottle, while tomato plants showed a general mild mosaic. It was, therefore, necessary to test thoroughly for virus *X* by grafts and inoculations to the potato varieties Arran Victory, President, Epicure and King Edward. Grafts to the first two produced top-necrosis, whereas inoculations gave black local lesions and no systemic symptoms. On Epicure and King Edward, in both of which the recognized strains of virus *X* produce top-necrosis, grafting and inoculation produced the same result, a definite mottling and deformity of the upper leaves, with some necrotic spotting on the lower leaves, which matured and fell early.

Thus, no evidence was obtained that virus *X* was present, and a large number of transmissions by different methods to many potato varieties gave no reason to suspect the presence of more than one virus or virus strain. The virus is readily transmitted by inoculation to tobacco, tomato and *D. stramonium*, as well as to potato varieties such as Majestic, Arran Consul and Arran Banner, in which it is carried or causes a mild mosaic, and to Epicure and King Edward, in which it causes a more severe mosaic. In varieties such as President and Arran Victory, in which grafting causes top-necrosis, inoculation never gave any symptoms except necrotic local lesions. The potato seedling U.S.D.A. 41956, which is immune to virus *X*, we were unable to infect with virus *B* either by grafting or inoculation.

These results differ from those of earlier workers, who believed they were working with pure cultures of virus *B*, and suggest that their cultures still contained virus *X*. Dykstra (1935, 1939) and Dennis (1939) recovered virus from seedling 41956 grafted with an Up-to-Date infected with *X* and *B*, and as this seedling was known to be immune to *X*, considered that this was *B* alone. Their cultures, however, as well as that of Clinch (1942) from an Up-to-Date she believed to contain only virus *B*, caused top-necrosis in both Epicure and King Edward, varieties which tolerate *B* but not *X*. Our results with seedling 41956 agree with Clinch's and at first sight are incompatible with those of Dykstra and Dennis, but there is no necessary contradiction, and the fact that they recovered virus from this seedling is not proof that it is susceptible. They grafted scions of Up-to-Date to seedling 41956 and then grafted scions from the 41956 to tomato and *D. stramonium*. Clinch (1942) showed that viruses *X* and *B* readily pass through a piece of seedling 41956 inserted as an intermediate scion between a scion of an infected variety and a susceptible stock, although she found no evidence that either virus could multiply in it. It seems likely that Dykstra and Dennis recovered both viruses from the 41956 plants

grafted with Up-to-Date scions, but that the virus particles recovered were merely some that had moved from the scion and not some produced by multiplication in the stock. After union between the scion and stock, there would presumably be a steady movement of virus particles into the stock, and, in the absence of direct inactivation by the resistant stock, these would be expected to remain infective for some days or even weeks.

The existence of a source of virus *B* free from virus *X* allowed their properties and immunological relationships to be tested. The results fully confirm Clinch's (1942) conclusion that they are related strains and not distinct viruses. No significant differences were found between the properties of the two in sap from infected tobacco. Both are inactivated by 10 min. heating around 68° C., lose their serological activity at approximately the same rate as their infectivity, and have a large temperature coefficient of inactivation. Both become inactive after 6–8 weeks' ageing *in vitro* at room temperature, and different samples of sap have dilution end-points varying from 1/10,000 to 1/50,000. Purification methods that are successful for virus *X* give good preparations of virus *B*; preparations of both viruses show anisotropy of flow and with antisera give fluffy open precipitates similar to those of bacterial flagellar antigens. Each virus is also precipitated by antiserum made against the other, and plants infected with one are protected against infection with the other. Table 1 shows the results of a protection test in which tobacco plants first inoculated with virus *B* were reinoculated with X^s, a strain giving countable necrotic local lesions. The left-hand halves of leaves were rubbed with sap containing virus *B* and the right-hand halves with water. After various intervals the whole leaves were rubbed with sap containing X^s and the lesions produced on the halves were counted separately. The average number of lesions on the halves first rubbed with water remained reasonably constant around 300 per leaf in all the tests, whereas on those first rubbed with virus *B* it fell rapidly as virus *B* multiplied. Similarly, when virus *B* was inoculated to President and Arran Victory plants infected with X^L, it gave no black necrotic local lesions such as it does in healthy plants.

These results disagree with those reported earlier (Bawden, 1936; Dennis, 1939), when viruses *X* and *B* were found to differ in their resistance to dilution and ageing, and plants infected with virus *X* were found to be still susceptible to virus *B*. The earlier tests were all made with inoculum containing both viruses, and this probably explains the differences found in the *in vitro* properties. As each virus inhibits the multiplication of the other, neither multiplies to its maximum possible concentration and, in the absence of deliberate selection, the proportions of the two tend to remain constant in successive hosts unless a host is used in which one multiplies more rapidly than the other. It seems likely that the mixture used in the earlier tests contained much more *X* than *B*, as the apparent differences lay in properties largely determined by concentration. The discrepancies between the earlier plant protection tests and those described here can be explained, as Clinch (1942) showed, by the different methods used. In the earlier tests, either potato plants infected with *X* were grafted with scions containing *B* or *D. stramonium* infected with *X* was inoculated with *B* and then grafted on to President potatoes. The introduction of grafting as a method of transmission accounts for the apparent failure to get protection, for we have again got similar results by repeating these experiments. Although potatoes infected with *X* developed top-necrosis when grafted with scions containing *B*, the disease was often less severe than when healthy stocks were used, a fact noticed earlier and suggested as possible evidence for protection (Bawden, 1936).

Table 1. *Protective effect of viruses B and X^a against X^s*

Interval between 1st and 2nd inoculation (days)	Relative nos. of X^s lesions on half leaves first rubbed with		
	Water	Virus *B*	Virus X^a
1	100	52	63
2	100	32	34
4	100	12	20
6	100	0·3	2
8	100	0·6	1·5

It seems that even when the second virus is introduced by inoculation, protection is often not absolute; e.g. plants infected with virus *B* usually give one or two necrotic lesions if rubbed all over with X^s. This fact probably accounts for the results with the reinoculated *D. stramonium*. The presence of virus *X* largely protects them from *B*, but the small amount that enters is sufficient to cause top-necrosis when scions are grafted to intolerant hosts. In these tests it was noticeable that the necrotic symptoms were usually much less severe than in plants of the same variety grafted with scions containing *B* alone.

Although all our tests show viruses *X* and *B* to be related strains, with presumably a common origin, we have been unable to confirm Clinch's suggestion that *B* is continually mutating to give rise to strains of virus *X* that do not produce top-necrosis in President and Arran Victory. The results she interprets in this way, we get only when using a mixture of the two virus strains. Then inoculated plants of intolerant varieties frequently develop a systemic mottle in addition to local lesions, and if plants infected by grafting leave any tubers that do not shrivel and die, these often give rise to new plants showing a mottle. Working with Scott's culture of virus *B*, we never obtained any mosaic symptoms in these two potato varieties. The fre-

quency with which systemic mosaic symptoms are obtained in President inoculated with a mixture of viruses *X* and *B* depends, in part at least, on the relative proportions of the two in the inoculum. If there is much more virus *B*, many necrotic local lesions are formed and it is rare to get a systemic infection with virus *X*, but if there is more virus *X*, there are few local lesions and more plants become systemically infected.

Most of the commonly occurring strains of virus *X* produce top-necrosis in Epicure and King Edward but not in President or Arran Victory—and so many strains, which vary in the type and severity of symptoms they cause in tobacco, are known with this property that it has almost become established as a criterion for virus *X*. The work with virus *B* described by Salaman (1938) as typical of X^g. From its properties *in vitro* and its immunological relationships with X^s, this virus is clearly an aberrant strain of virus *X* and we shall refer to it as X^a. It reacts with antiserum prepared against X^s or *B*, and its ability to protect tobacco plants against X^s is shown in Table 1. There are also other strains of virus *X* that differ in their reactions on potato varieties from any previously described: e.g. from a Majestic plant in the field suffering from top-necrosis we obtained a virus serologically related to X^s and *B*, which killed Majestic with top-necrosis while causing only a mottle in President.

Straightforward precipitation tests show no serological differences between these *X*-type viruses, for all behave in much the same way whether titrated against their homologous or heterologous antisera. However, they are not antigenically identical, for differences can be demonstrated by cross-absorption tests. We have not attempted a detailed antigenic analysis, but the summarized results in Table 2 show that the individual strains contain specific antigens and suggest that the greater the differences in the symptoms caused the greater are the antigenic differences. For example, X^s and X^y, two strains that differ mainly in the severity of symptoms caused in tobacco, differ from each other less than they differ from X^a or *B*.

TABLE 2. *Cross-absorption experiments with strains of virus X and their antisera*

Absorption		Reaction of absorbed serum with			
Serum absorbed	Antigen used	X^s	X^y	X^a	*B*
X^a	X^s	−	−	+	−
X^a	*B*	−	−	+	−
X^a	X^y	+	−	+	+
B	X^s	−	−	+	+
B	X^a	−	−	−	+
B	X^y	+	−	+	+
X^s	X^a	−	−	−	−

Before each absorption, the optimal combining proportion of the antigen and serum to be used was determined: 1 c.c. of antigen at varying concentrations was added to each of a series of tubes containing 1 c.c. of serum at a constant dilution, and the proportions of antigen and serum at which precipitation first occurred were taken as optimal. Antiserum and antigen were mixed with antigen at twice the optimal value. After 2 hr. incubation at 50° C., the mixture was left overnight at 0° C. and then centrifuged. The supernatant fluid at a constant dilution was tested against the antigen used for absorption over a wide range of antigen dilutions. If there was no precipitation, the absorbed sera were tested for their ability to precipitate other virus strains, tests being made with constant serum dilution and with antigen varying over a wide range of dilutions. A + indicates that the absorbed serum precipitated the particular virus strain and a − that it did not.

shows clearly that such a simple test based on host reactions is not a valid test of relationships. This fact is emphasized by work with another virus supplied to us by Mr Scott, who suspected it to be a strain of virus *X* although it failed to cause top-necrosis in any potato variety he infected. In Epicure and King Edward it causes a mottling and crinkling of the upper leaves, while the lower leaves develop necrotic spots, mature early and fall, the whole effect resembling that caused by virus *B*. President and Arran Victory, in our glasshouses, act as symptomless carriers of this virus, and infected tobacco plants show a faint, spotty, interveinal mottling of the type

Intracellular inclusions

Salaman (1938) found that all his strains of virus *X* produced intracellular inclusions in infected tobacco and *D. stramonium*. We have examined tobacco and tomato infected with all the strains discussed here, as well as the potato vars. President and Majestic with X^y, President with X^L, Arran Victory with X^a, and Duke of York and King Edward with *B*. We found inclusions in all the infected hosts we studied except the last two, but the frequency with which they were found varied with the different strains and with the host plant. In general, it seems that the production of a bright yellow mosaic with little or no necrosis favours the maximum production of inclusion bodies. Salaman's X^L, and the strain we called X^y, which also produces a general interveinal mosaic in both tobacco and President potatoes, produces far more inclusions than X^s, a strain causing necrotic rings in tobacco, or than the avirulent strain X^a. External symptoms are not an absolute guide, for virus *B*, which also gives a yellow mosaic in tobacco, does not produce large numbers of inclusions.

Unlike the inclusions of tobacco mosaic virus, which occur mainly in the epidermis and hair cells, most of those produced by *X*-type viruses are in the palisade and spongy parenchyma. In living material they are less easy to distinguish from the cytoplasm than those of tobacco mosaic virus, but the presence of oil globules and doubly refractive particles often

helps in identification. In fixed preparations, the oil may be removed and the inclusions appear vacuolate and granular. They give the usual protein reactions, but do not stain with the Feulgen technique and so can be readily distinguished from the nuclei.

In tobacco the inclusions produced by all strains of *X* are usually elliptical and only slightly larger than the nuclei (Pl. 3, fig. 3). In potato they may be ×6 or more the size of the nucleus and occupy as much as half the volume of the cell; unless compressed by the cell walls they are roughly spherical. When first formed they are colourless, but later become yellow (Pl. 3, fig. 4).

It was more difficult to follow the method of formation of these inclusions than those of the aucuba strain of tobacco mosaic virus (Sheffield, 1931), because of the greater difficulty in differentiating the material forming inclusions from the normal cytoplasm. It seems likely that they are formed in a similar manner by the gradual aggregation of small particles. When symptoms first become noticeable in infected tobacco leaves, the cytoplasm streams more rapidly, and becomes more obvious and apparently more abundant than in healthy cells of the same age. Unusually wide bands of cytoplasm are formed, which bulge into the vacuole and can be seen to increase in size as they flow around the cell. Similar aggregations often occur at a junction where several cytoplasmic strands meet, or at a corner of a cell or even in indentations on the nucleus (Pl. 3, fig. 7). These aggregations sometimes break down, but often become rounded into a body and retain their identity as long as the cell can be kept alive and under observation. Similarly, in infected potatoes before fully formed inclusions are found, irregularly shaped pieces of material, distinguishable from the normal cytoplasm, can be seen moving slowly around the cell. It seems likely that these are produced by aggregation of still smaller particles and themselves ultimately fuse to give the large inclusions.

The inclusions formed by the aucuba strain of tobacco mosaic virus (Sheffield, 1939) disintegrate rapidly if pricked or cut, but those formed by *X*-type viruses are more resistant to rough handling. Inclusions have been removed from cells of tobacco plants infected with X^L and they retain their individuality in 0·1 *M* phosphate buffer at *p*H 7. These can be repeatedly cut into small fragments without dissolution.

Inclusions in the epidermal layers appear to be equally distributed in the green and yellow parts of affected leaves, whereas in the palisade cells and spongy parenchyma they are usually restricted to the yellow areas. With X^v and X^L, which produce large chlorotic areas, pieces of potato leaf 2–3 mm. long are often found in which almost every cell contains an inclusion. With X^a also, patches occur in which each cell contains an inclusion, but these are smaller, rarely exceeding ten cells. Obvious external symptoms are not essential for the production of inclusions, as we have found them in Arran Victory infected with X^a and such plants in our conditions are externally indistinguishable from uninfected plants. Virus *B* appears to produce fewer lesions than the other strains of *X*. We have found some in infected tobacco plants, but not in Duke of York or King Edward potatoes, although the former showed a definite interveined mottle and the latter a severe mosaic.

Clinch (1932) found a few striated masses, similar to those produced abundantly by tobacco mosaic virus in living cells of potatoes with crinkle or interveinal mosaic. In fixed preparations she occasionally found one or two long filiform bodies. In a few cells of a single living tomato leaf infected with X^L virus we found numbers of long, fibrous, doubly refractive bodies (Pl. 3, fig. 8). These were similar in form to inclusions described by Kassanis & Sheffield (1941) in plants infected with various strains of tobacco mosaic virus, but were darker in colour. No virus other than X^L could be recovered from this plant.

Many binucleate cells were found in all hosts infected with these strains of virus *X*. Degeneration of nuclei was seen only in plants infected with strains causing necrotic symptoms, and seemed to be a prelude to necrosis.

Viruses *A*, *C* and *Y*

When sap from the Edgecote Purple plant, supplied by Mr Scott as infected with virus *C*, was inoculated to tobacco plants, these developed symptoms characteristic of infection with virus *Y* (Pl. 3, fig. 1). Ten days after inoculation the young leaves showed a clearing of the veins, which faded in a week or so to be succeeded by the formation of dark green bands along the veins. The symptoms developed more slowly and were rather less severe than those caused by our culture of virus *Y*, but they were of essentially the same type. Sap taken from these plants was precipitated specifically by virus *Y* antiserum, although it gave a lower precipitation end-point than sap infected with virus *Y*. Grafts from the Edgecote Purple plant to President, Up-to-Date, Majestic and Arran Victory showed the presence of virus *C*, for the first three died of top-necrosis while the fourth developed only a mosaic. Two explanations of these results seemed equally possible: either virus *C* was a strain of virus *Y*, or the Edgecote Purple plant was infected with a mixture of viruses *C* and *Y*. All our results support the first, and we were unable to get any evidence that the Edgecote Purple plant contains more than virus *C*.

We inoculated sap from plants infected with virus *C* to many potato varieties in which virus *Y* causes the characteristic disease leaf-drop streak (acropetal necrosis), but consistently failed to get any systemic infection, although control plants inoculated at the

same time with a mixture of sap from plants infected separately with *Y* and *C* all developed leaf-drop streak. Similarly we failed to transmit any virus by means of *Myzus persicae* from plants infected with virus *C* in conditions in which 100% transmission of virus *Y* was obtained. Tests on resistance to heating and ageing also suggested that only one virus was present; in these, virus *C* behaved very like *Y*, being inactivated in tobacco sap by 10 min. at 57° C. or by a week at room temperature.

Further evidence that virus *C* is a strain of *Y*, in spite of the strikingly different symptoms they cause in many potato varieties and their differences in mode of transmission, was supplied by plant protection tests. Ten tobacco plants were inoculated with virus *C*, and a month later, when they were showing vein-banding symptoms, these were re-inoculated with virus *Y*. At the same time ten healthy plants of the same age were also inoculated with virus *Y*. The plants first inoculated with virus *C* developed no new symptoms, whereas the previously healthy plants showed first a vein-clearing and then vein-banding. A month after the second inoculation, sap from each of the plants was inoculated to two Majestic plants. All the inoculated potato leaves developed similar black necrotic local lesions; this was the only symptom on those inoculated with sap from tobacco plants first infected with virus *C*, whereas all those inoculated with sap from plants with *Y* alone developed leaf-drop streak.

There are previous claims that potato virus *Y* exists in strains causing widely different diseases: e.g. Chester (1937) considered that virus *Y* is serologically related to cucumber virus 1 and Hansen (1937) that it is serologically related to potato virus *A*. We were unable to confirm these results. Sap from plants infected with either of these two viruses failed to precipitate specifically with virus *Y* antiserum. This is not conclusive evidence that the viruses are not related, for we have not prepared antisera against cucumber virus 1 and virus *A* and shown that these viruses under the conditions of our tests are precipitated by their homologous antisera. Thus, the absence of precipitation could be merely the result of a too small content of virus in the preparations tested. Another reason for regarding these as separate viruses, even though they have many similar properties, is that they do not protect plants against one another. Tobacco plants infected with either virus *A* or cucumber virus 1 are as susceptible to potato virus *Y* as are healthy plants. It is likely that at least Chester's results were caused by the use of a mixture of viruses either as a test or an immunizing antigen, for the clinical effects he attributes to potato virus *Y* differ in many ways from those found by other workers. Variants of virus *Y* that produce less severe symptoms than the normal form in both tobacco and potato were described by Salaman (1937) and Köhler (1937). That such variants occur frequently in the field is suggested by the wide range of symptoms shown in the second year of infection by individual plants of varieties showing leaf-drop streak in the first year of infection.

We did not determine the reactions of virus *C* on a wide range of potato varieties, but such tests as we made suggest that virus *C* produces top-necrosis when transmitted by grafting to the varieties in which *Y* causes acropetal necrosis. In such varieties, both viruses may give identical local lesions (Pl. 3, fig. 2), but only *Y* becomes systemic by inoculation. In varieties such as Arran Victory, in which *Y* produces only mosaic symptoms, *C* also causes a mosaic and to these it is readily transmitted by inoculation. It can be transmitted to tomato as easily as to tobacco by inoculation: *D. stramonium* seems to be immune to virus *C* in addition to *Y*. We were unable to confirm the statement of Salaman & Wortley (1939) that cabbage and Brussels sprouts are susceptible to virus *Y*, and failed to infect these plants with *Y* or *C* by inoculation or with *Y* by means of *Myzus persicae*.

Intracellular inclusions

Previous workers could not find any intracellular inclusions in plants infected with virus *Y* and it was concluded that this virus does not produce them (Bawden, 1939). We have now found such bodies, though rarely, in both potato and tobacco plants infected separately with viruses *C* and *Y*. These virus strains produce the same type of inclusion and, unlike the *X*-type viruses, produce the same type in potato as in tobacco (Pl. 3, figs. 5 and 6). They are small and inconspicuous; in tobacco they resemble those caused by *X*-type viruses, but the two cannot be confused in potato where the *X* viruses produce many large inclusions. Inclusions were found in President, Majestic and King Edward plants infected with *Y* in the current season as well as in plants raised from infected tubers, and in Edgecote Purple plants infected with virus *C*. Inclusions were seen in all tissues of the leaves except the vascular strands, but they occur so infrequently that often many sections must be searched before one is found. No inclusions were found in tobacco plants infected with virus *A*.

Discussion

This work on potato viruses shows the dangers attending the use of symptomatology as a basis for grouping and classifying viruses. The clinical picture produced by infection is as much a property of the host as of the virus, and related strains may produce widely different diseases in the same host while unrelated viruses may cause identical symptoms: e.g. in Majestic potatoes one strain of virus *X* causes top-necrosis and another a mottle, while one strain of virus *Y* causes top-necrosis and another acropetal necrosis. For distinguishing between viruses a study

of the symptoms caused on a series of differential hosts is of great value, as two viruses producing different symptoms on the same hosts cannot be identical. Such a study, however, cannot tell whether the two are related strains or not: for this, the properties of the viruses *in vitro* and their immunological reactions must be examined. The ability of one virus to protect plants against another appears to be one of the most convenient tests for demonstrating close relationships between viruses transmitted by inoculation, but is less reliable when grafting has to be used as a method of transmission. Methods of transmission also appear to be of little value as a basis for establishing relationships, as one strain of a virus may give systemic infection when inoculated to a particular host and a second may not. Also, our work with viruses *Y* and *C* shows that one strain of a virus may be readily transmitted by insects which will not transmit another.

The fact that viruses producing widely different symptoms in the same host may be related strains, and so presumably originate from one another by a process akin to mutation, raises important practical problems. Most commercial potato varieties that tolerate infection with the common forms of virus *X* are almost 100% infected, and their yield is reduced by about 20% (Scott, 1941; Bald & Norris, 1941), whereas the health of varieties such as King Edward and Epicure, which die when infected, is much higher (Cockerham, 1941). Already we know two strains of *X* that do not kill these varieties and both have been found in King Edward in the field. Fortunately, these strains produce such severe symptoms that roguing is moderately easy, but the arising of other strains causing less definite symptoms is an obvious possibility. The establishment of such strains in these varieties would be equivalent to the introduction of a new disease, and would soon lower their standard of health to that of the other varieties. One of the most promising methods of controlling the losses caused by mild mosaic is to breed varieties that react with top-necrosis when infected with the commoner viruses and virus strains, as such varieties rogue themselves most efficiently. No such variety, however, should be regarded as a permanent solution to the problem. There is good reason to believe that viruses constantly change and the forms that persist are likely to be those causing less severe diseases, for with obligate parasites such as viruses, the ability to survive depends on the existence of hosts which survive when infected. Selection continuously operates against the viruses causing lethal diseases, and the strains of *X* most widely distributed are those, such as X^y or X^L, which cause top-necrosis in only a few cultivated varieties. With increase in the number of intolerant varieties, selection will operate against these strains in favour of any that do not cause top-necrosis, unless additional precautions are taken to ensure that these are excluded from the stocks. In addition to the breeding of hypersensitive varieties, therefore, there is need for further work on the methods of spread of these viruses and for the elaboration of other control measures.

Note added in proof: The reactions of 146 potato varieties to viruses *X*, *A*, *B* and *C* have been described by Cockerham (*Ann. app. Biol.* 1943, **30**, 338).

REFERENCES

Bald, J. G. & Norris, D. O. (1941). The effect of the latent virus (virus *X*) on the yield of potatoes. *J. Coun. sci. industr. Res. Aust.* **13**, 252–4.

Bald, J. G. & Pugsley, A. T. (1941). The main virus diseases of the potato in Victoria. *Pamphl. Coun. sci. industr. Res. Aust.* **110**, 1–40.

Bawden, F. C. (1936). The viruses causing top-necrosis (acronecrosis) of the potato. *Ann. appl. Biol.* **23**, 487–97.

—— (1939). *Plant Viruses and Virus Diseases.* Leiden: Chron. Bot.

Chester, K. S. (1937). Serological studies of plant viruses. *Phytopathology*, **27**, 903–12.

Clinch, Phyllis E. M. (1932). Cytological studies of potato plants affected with certain virus diseases. *Sci. Proc. R. Dublin Soc.* **20**, 143–72.

—— (1942). The identity of the top-necrosis virus in Up-to-Date potato. *Sci. Proc. R. Dublin Soc.* **23**, 18–34.

Cockerham, G. (1939*a*). The distribution and significance of certain potato viruses in Scotland. *Scot. J. Agric.* **22**, 1–11.

—— (1939*b*). *Rep. Scot. Soc. Res. Pl. Breed.* pp. 19–21.

—— (1941). *Rep. Scot. Soc. Res. Pl. Breed.* pp. 8–13.

Dennis, R. W. G. (1939). Studies on *Solanum* virus 4. *Phytopathology*, **29**, 168–77.

Dykstra, T. P. (1935). A top-necrosis virus found in some apparently 'healthy' potatoes. *Phytopathology*, **25**, 1115–16.

—— (1939). A study of viruses infecting European and American varieties of the potato, *Solanum tuberosum*. *Phytopathology*, **29**, 40–67.

Hansen, H. P. (1937). Studier over Kartoffelviroser i Danmark. *Tidsskr. Planteavl.* **42**, 631–81.

Kassanis, B. & Sheffield, F. M. L. (1941). Variations in the cytoplasmic inclusions induced by three strains of tobacco mosaic virus. *Ann. appl. Biol.* **28**, 360–7.

Köhler, E. (1935). Mischinfektionen mit verschiedenen Stämmen des Ringmosaikvirus (*X*-virus Gruppe) der Kartoffel. *Angew. Bot.* **17**, 60–74.

—— (1937). Zur Frage der Schutzimpfung bei den Veinbanding-Viren (Vorläufige Mitteiling). *NachrBl. dtsch. PflSchDienst.* **17**, 32–3.

Quanjer, H. M. (1931). The methods of classification of plant viruses, and an attempt to classify and name potato viruses. *Phytopathology*, **21**, 577–613.

Salaman, R. N. (1937). Acquired immunity against the '*Y*' potato virus. *Nature, Lond.*, **139**, 924.

—— (1938). The potato virus '*X*': its strains and reactions. *Philos. Trans.* B, **229**, 137–217.

SALAMAN, R. N. & WORTLEY, W. R. S. (1939). Potential hosts of potato viruses in garden and field. *Nature, Lond.*, **144**, 1049.

SCOTT, R. J. (1938). Mosaic diseases of the potato. *Scot. J. Agric.* **21**, 121–32.

—— (1941). The effect of mosaic diseases on potatoes. *Scot. J. Agric.* **23**, 1–7.

SHEFFIELD, F. M. L. (1931). The formation of intracellular inclusions in solanaceous hosts infected with aucuba mosaic of tomato. *Ann. appl. Biol.* **18**, 471–93.

—— (1939). Micrurgical studies on virus infected plants. *Proc. roy. Soc.* B, **126**, 529–38.

EXPLANATION OF PLATE 3

Figs. 3–8 are photomicrographs taken with a Leitz Makam camera. Figs. 3–6 are sections of leaves fixed in Zenker's solution and Figs. 7 and 8 are living cells. For the photographs a Leitz × 10 periplanat ocular was used in combination with a Leitz apochromatic 2 mm. objective (N.A. 1·4) (Figs. 3–6) or a Leitz 3 b (Fig. 7) or a Leitz 6 L (Fig. 8) objective.

Fig. 1. Leaf from tobacco plant infected with virus *C* showing vein-banding symptoms: photo. 6 weeks after infection.

Fig. 2. Leaves of Majestic potato showing necrotic local lesions caused by virus *Y* (*a*) and virus *C* (*b*).

Fig. 3. Tobacco infected with virus X^{v}. A palisade cell with a small vacuolate inclusion body (Heidenhain's haematoxylin, × 900).

Fig 4. Potato var. President infected with virus X^{Y}. The palisade and spongy parenchyma contain many large inclusions (Feulgen and light green, × 900).

Fig. 5. Potato var. King Edward infected with virus *Y*. A small vacuolate inclusion body lies near the nucleus of a parenchymatous cell (Heidenhain's haematoxylin, × 900).

Fig. 6. Potato var. Edgecote Purple infected with virus *C*. An epidermal cell contains a nucleus and a small inclusion body (Heidenhain's haematoxylin, × 900).

Fig. 7. Tomato infected with virus X^{L}. Some of the cytoplasmic material is forming into clumps (unstained, × 140).

Fig. 8. Tomato infected with virus X^{L}. A hair cell contains fibrous inclusion bodies (unstained, × 450).

(*Received* 19 *April* 1943)

The 'phloem necrosis' virus disease of tea in Ceylon

I. Introductory account, symptoms, and transmission by grafting

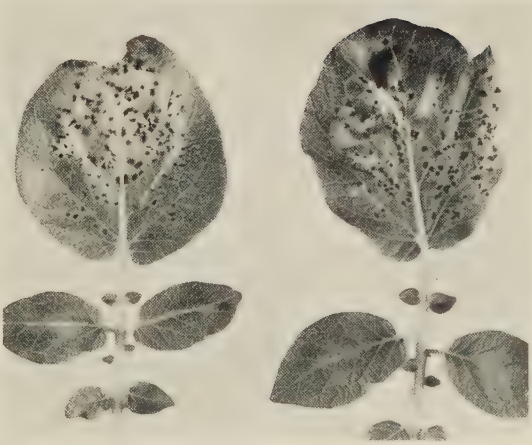
BY T. E. T. BOND, *Tea Research Institute of Ceylon, St Coombs, Talawakelle*

(With Plates 4 and 5)

A virus disease of tea in the up-country districts of Ceylon is described which is known as 'phloem necrosis' from the characteristic internal symptoms. External symptoms including leaf curl and zigzag and dwarfing of the shoots are of less constant occurrence and appear to be much influenced by climatic and growth conditions generally. Severely affected bushes are rendered entirely unproductive. The disease is shown to be transmissible by grafting to certain types of tea, nine clones of various origin having been infected so far. In others, of the type commercially favoured for replanting, no symptoms are produced. These have proved to be symptomless 'carriers' capable of transmitting the disease to further susceptible scions. The theoretical aspects of the results are discussed, considerations of control and the practical significance of the 'carrying' capacity being left for a further paper.

'Phloem necrosis' is a serious disease of tea in the up-country districts of Ceylon. It has been under investigation by the Tea Research Institute since the end of 1935: the writer took over this problem on his appointment as Research Officer in February 1940. The disease was described by Gadd (1939*a*, *b*) in the Institute's quarterly journal, and summaries of investigations in progress have appeared in the Institute's Annual Reports from 1936 onwards. Although the investigations are still incomplete, there is now enough material at hand to commence publication in a technical journal. The present paper is intended to provide a general introductory account of the disease and its symptoms and to give the results of graft transmission experiments whereby its virus nature is established and the occurrence of 'carrier' varieties demonstrated. Other aspects will be dealt with subsequently as data become available.

Fig. 1



(*a*) (*b*)

Fig. 2

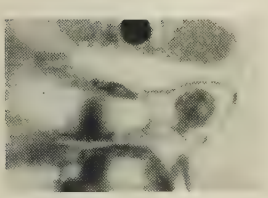


Fig. 3

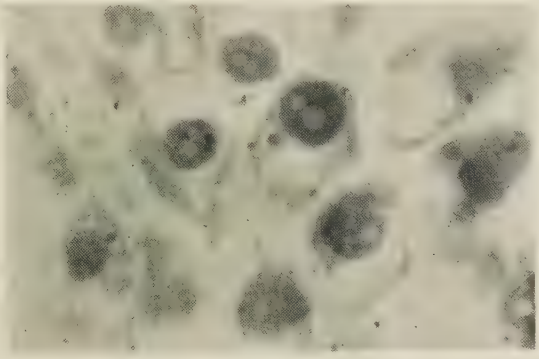


Fig. 4

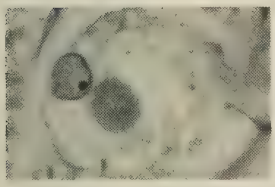


Fig. 5

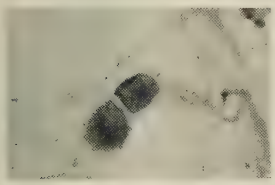


Fig. 6

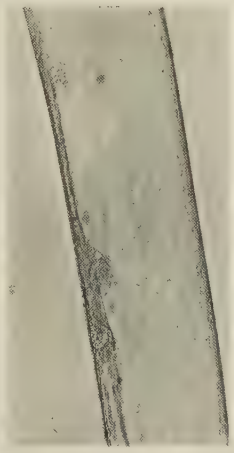


Fig. 7

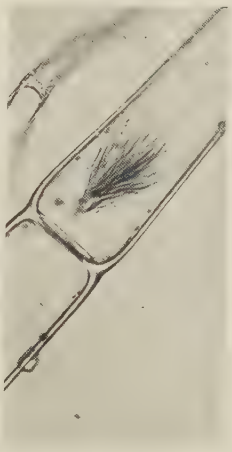


Fig. 8

Terminology

The following terms applicable to the growth of the tea bush in Ceylon need brief definition only: fuller treatment of some has already been given (Bond, 1942).

Flush and *banji* respectively refer to the shoot as undergoing active growth, with the production of new leaves, and as apparently dormant, increasing passively in size to the maximum and with subsequent lignification of the tissues. The *pruning cycle* is the interval between prunings; up-country it normally lasts 3–5 years, the bushes being pruned down to within 1 ft. or so from the ground. The new shoots are then *tipped* or headed back once or twice before normal plucking again commences.

Jât. Owing to intense hybridity, tea is an extremely variable plant and most estates show a great diversity of type. Generally speaking, the larger-leafed bushes are classed as 'high *jât*' and the smaller-leafed as 'low *jât*'. Deterioration of *jât* implies a gradual loss of vigour and productivity, with a reduction in average leaf size. This is not to be confused with the normal tendency for the average leaf size to decrease during the pruning cycle from tipping onwards. Much of the older tea at high elevations is of relatively 'low *jât*', whereas the modern tendency in replanting is to use only 'high *jât*' supplies.

History and distribution

The original cases of 'phloem necrosis' were reported from a single planting district in the centre of the up-country zone and including most of the highest tea estates in the Island, nearly all above 6000 ft. In this district the disease proved to be of general occurrence and appeared to have existed for many years: it was increasing slowly and was the cause of considerable loss. During 1938 it was reported from another estate in a different district where, again, it was believed to be of long standing. It was afterwards discovered on the Tea Research Institute's own estate, and adjoining properties, where the elevation was 4–5000 ft. only. In 1939 the disease was given wide publicity at a Planters' Conference and elsewhere and the result was a big increase in the number of cases reported. It was evident that this had little, if any, connexion with a natural increase in range of the disease. At the present time (June 1943), 'phloem necrosis' is known for certain from 117 estates representing a total of 75,730 acres actually under tea. Of these estates, only five are below 4000 ft. (the lowest at 3400 ft.) and very nearly half, representing 31,413 acres, are above 5000 ft. It seems likely, from the available records, that the disease occurs almost universally above 4000 ft. and that above 5000 ft. it is potentially, and above 6000 ft. distinctly, of importance. In all, the acreage affected is probably twice as great as that actually reported, or about 150,000 out of a total acreage under tea in Ceylon of some 550,000 acres.

Symptoms and diagnosis

The full symptom expression in 'phloem necrosis' is unusually complex, and accurate diagnosis is in many cases difficult. Before the disease was recognized as such, affected bushes had been regarded merely as unproductive and of rather 'low *jât*' character. Such bushes had for some years been systematically cut out, yet it appeared that the type had not been eliminated but rather showed a tendency to increase. Hence it was naturally imagined by planters that a deterioration of *jât* had set in, perhaps as a result of continued growth at the high elevation of the district chiefly concerned. The distribution of these unproductive bushes in the field was suggestive of a root disease and the first specimens received for diagnosis were examined from that point of view. Thus the earliest definite symptom to be recognized was the occurrence of necrosis affecting the phloem of the roots—hence the name 'phloem necrosis' given to the disease in 1936. Later the necrosis was found to affect the phloem in the aerial parts of the plant as well. Meanwhile, it was gradually realized that many necrotic bushes could also be recognized by the occurrence of one or more externally visible symptoms of which a tendency for the leaves to curl and for the stems to develop in a zigzag fashion was the most important. The main symptoms, both internal and external, are discussed in the ensuing paragraphs.

Necrosis in the root and stem. The occurrence of necrosis in the root appears to be the most constant single diagnostic feature of the disease. There are no external lesions on affected roots, but the necrosis is readily demonstrated by shaving away a slice of bark so that the phloem is exposed close to the cambium (Pl. 5, fig. 1). Usually, the bark is considerably thicker than in the normal healthy root. The necrosis varies in appearance, according to the severity of the infection, from a minute, scarcely visible, flecking to a prominent and irregular spotting or blotching. In the stem, the presence of necrosis can be detected most easily by peeling a strip of bark and viewing the exposed inner surface of the phloem removed with the strip.

Necrosis in the leaf. This can usually be seen with the naked eye in the cut or broken end of the petiole, and if severe enough it will impart a brown discoloration to the entire vein system as viewed from the lower surface of the leaf. The position of the necrotic tissue in transverse sections of the petiole or midrib bundle is important: it should be peripheral in origin, lying on the outer edge of the phloem and close within the pericycle (Pl. 5, fig. 2). In young plants, and in experimental material

generally, some difficulty has been caused by the spontaneous occurrence of a 'false necrosis' affecting the inner region of the phloem and with a marked tendency to radial development in association with the 'medullary' rays (Bond, 1942). The cause of this 'false necrosis' has not been determined, but there is no evidence to suggest that it is associated with 'phloem necrosis' proper. Further work on the precise characterization of the different types of necrosis is in progress.

Leaf curl and 'zigzag'. Typical symptoms affecting the aerial shoots are illustrated in Pl. 4. The curling of the leaves takes the form of a backward arching, in mild cases often associated with an inward folding of the lamina along the midrib (fig. 3) and when severe resulting in a twisting and crumpling of the apical portion of the leaf (figs. 4–5). Badly affected shoots are much dwarfed and thus contrast with others in which the symptoms are less pronounced (fig. 1). The severe curl is usually associated with a zigzag habit of growth: both symptoms are seen most prominently in the dwarfed regions, but either may develop, to a certain extent at least, independently of the other (figs. 3–6). The situation is also complicated by the fact that varying degrees of natural leaf curl appear characteristic of certain *jâts* in the healthy condition. A zigzag or undulate habit of growth is also noticed occasionally in otherwise healthy tea and, in Java, this condition was described by Wellensiek (1938) as an inherited variation of possible value for the ready identification of certain clones. The most typical form of pronounced leaf curl and zigzag, with dwarfing, is not, however, likely to be confused with any normal modification of growth occurring in healthy tea, although there is a resemblance in young shoots of the type shown in fig. 5 between severe leaf curl and certain forms of aphis injury. In the latter case, the occurrence of necrosis is diagnostic of the virus disease.

Leaf curl, zigzag, and dwarfing can usually be attributed, histologically, to the premature lignification of the pericycle in the midrib of the leaf and the leaf trace below. Normal lignification occurs only after the leaf and internode have reached their maximum size (Bond, 1942), and premature lignification of the pericycle would tend to arrest growth on the abaxial side of the leaf and leaf-trace system, the continued growth adaxially producing curling and distortion of the type described. Such a process would appear to be related to the carbohydrate balance in the leaf and the effect of 'phloem necrosis' on translocation, but no such relationship has as yet been determined. There is, for instance, no obvious excess of starch in the diseased tissues. Any theory which may be developed along these lines will also need to take into account the frequent presence of more or less severe necrosis without any associated external symptoms.

So far, in the field, no young plants or 'supplies' have been diagnosed with certainty as necrotic. The tea in the affected areas is mostly fairly old—from at least 30 to about 60 years—and the necrotic bushes are no exception to the general rule. Hence the development of symptoms in mature bushes and in young, experimental material presents distinctive problems.

Diagnosis in the field is ideally carried out by root examination, but in practice the more rapid method of leaf examination is usually adopted. The association between curl and other external symptoms and necrosis in the leaf is not high: only about half the necrotic bushes are externally recognizable in most areas. Where the disease is relatively severe, and at the higher altitudes generally, the proportion may be about three-quarters, although this is still low when compared with certain batches of young necrotic cuttings which may be curled throughout. External symptoms of 'phloem necrosis' are most in evidence shortly after pruning. This is due partly to the fact that the recovery from pruning of affected bushes is usually considerably delayed, and partly to the greater contrast between the dwarfed and normal shoots at this time. There is also a tendency for the bush to 'grow away' from the symptoms in the manner described below. One of the most confusing features of the disease in mature bushes is that the symptoms may be restricted to part of the bush only. Commonly a visibly necrotic root sucker may be found attached to a healthy, normal bush, or a single branch of the main shoot system may be affected. In all probability two distinct tendencies are involved here, namely a tendency for the virus to become concentrated in the roots and to move with difficulty thence to the main shoot system, and the possibility that movement from one branch, originally infected, to other parts of the plant is also a slow and gradual process. Both of these suggestions find a parallel in recent contributions by Fulton (1941) and Blodgett (1943). In a few instances, as in the bush illustrated in Pl. 4, fig. 2, the effect can be traced to what is apparently a natural graft union between two different bushes, perhaps derived from the germination of two seeds in the same hole. This bush appeared to spring from a single stem which, on sectioning, was found to consist of two closely adpressed tap-root systems joined by a narrow line of fusion. While it might be argued that the absence of symptoms in the 'healthy' component was temporary only, on account of restricted migration of the virus, it seems more probable that here a real difference in genetic constitution exists as affecting the reaction of the two components to infection.

More detailed information as to the course of symptom expression in the individual shoot was obtained by examining shoots of known necrotic origin, leaf by leaf and in each internode of the stem. To summarize, the *banji* shoots in the fully lignified

state (i.e. just before the commencement of the new flush period) may be necrotic up to the base of the apical bud. Necrosis-free gaps occur commonly and may persist, apparently, indefinitely. There is a general tendency for necrosis to occur at the base of the buds, even when these are subtended by a leaf in which no necrosis can be demonstrated. Necrosis occurred rather more frequently in the internodes (64%) than in the leaves (46%), but there was a distinct association between the condition of the leaf and that of the internode below it. This is shown by the following results obtained from an examination of 470 leaves and internodes from a total of eighty-two shoots. (The numbers expected on a purely random association are in brackets.)

	Petiole			
Internode	Necrotic	Not necrotic		
Necrotic	179 (138)	123 (164)	302	
Not necrotic	35 (76)	133 (92)	168	$\chi^2 = 64{\cdot}2$
	214	256	470	

With the commencement of the flush period, there is at first a tendency to 'grow away' from the necrosis, which is to be found towards the base of the new flush only, i.e. in the 'fish' leaves and below. Later, as elongation is completed with the onset of the subsequent *banji* stage, necrosis appears again throughout the shoot. There is no regular spread from the base: it is as commonly found first below the apical bud as in one of the middle internodes. At this stage there is a strong association between the occurrence of necrosis in the internodes and the lignification of the pericycle, viz.

	Internode			
Pericycle	Necrotic	Not necrotic		
Lignified	59 (43)	3 (19)	62	
Not lignified	5 (21)	26 (10)	31	$\chi^2 = 61{\cdot}3$
	64	29	93	

In the young flush shoot, symptoms of leaf curl and zigzag may precede the appearance of necrosis. Thus, in a batch of flush shoots of the type shown in Pl. 4, fig. 5, where the leaves were curled as soon as they were unfolded from the bud, necrosis was absent on the average from the first four or five leaves and internodes below the bud. A tendency to lignification of the pericycle was noticed in all the leaves, but was absent on the average from the first three internodes.

The degree of variability in symptoms capable of expression in a single shoot system is illustrated in Pl. 4, fig. 7. The main stem of this specimen was about a yard long and was actively flushing: it was severely necrotic internally, both in petioles and internodes, to within a couple of leaves of the apex. Several laterals were removed before the photograph was taken and these, like the main stem, showed various combinations of apparently healthy and severely diseased growth and were necrotic practically throughout. The large lateral of entirely healthy appearance on the right was necrotic only in the four lowest internodes. It consisted of a single flush period and had probably developed rapidly some months previously and remained *banji* ever since. The swollen condition of the apical bud indicated that a new flush period, corresponding to the current flush of the main shoot, was about to commence. The specimen was collected after a period of drought when, with the coming of the rain, a renewed rush of growth was starting: it suggests most strongly that the production of the dwarfed, curled and zigzag regions coincided with the drought and that a more healthy type of growth is produced when conditions are favourable. A point of considerable interest and significance is that single-node cuttings propagated from the 'healthy' and 'diseased' portions of this specimen were indistinguishable, and were all severely diseased. Thus, it appears that the virus is truly systemic and that climatic 'masking' of its effects may occur to an unusual degree.

Graft transmission experiments

Methods

Three of the most successful types of grafts are illustrated in Pl. 5, fig. 3. These and certain others are briefly noted below, following Garner's (1940) terminology as far as possible.

'*Seedling grafts*.' A method of side-grafting, by inserting a single-node cutting scion, with leaf attached, into a slice in the hypocotyl of a 1–3-month seedling (fig. 3, *centre*). The grafts are kept in peat after tying and are usually ready for transfer within 3 months.

'*Inarches*.' The usual method of approach grafting was followed.

'*Root grafts*.' Whip-and-tongue grafts between a single-node cutting and a portion of detached root (fig. 3, *right*), kept in peat usually for 3 months.

'*Couple grafts*.' The method of van Emden (1940) was followed (fig. 3, *left*). This is really a grafting process analogous to the 'Modified Forkert' system of budding, and is probably the most successful for use in the field.

'*Crown grafts*.' A few grafts by Keuchenius's (1923) method were tried, but without success.

Tea has proved a difficult subject for grafting and the percentage of success has been very variable. Under up-country conditions (4500 ft.) the union is in any case slow and the writer's experience indicates that the grafts are much subject to loss by drought and other unfavourable weather conditions for about 2 years afterwards. The grafts when potted or planted out were given no special protection against chance infection through the agency of insects nor was any

feasible under the circumstances. Adequate control was afforded by the proximity of many dozens of healthy cuttings of various clones, including those afterwards shown to be susceptible, in none of which were any indications of natural infection recorded during the course of the experiments.

Early experiments

Up to the end of 1939, nearly 600 grafts, mostly seedling grafts and inarches, had been made in the attempt to transmit 'phloem necrosis' to the ordinary, 'high *jât*' type of commercial planting material. These grafts have played an important part in subsequent developments and their history is given fairly fully.

The seedling grafts, which consisted of healthy seedling stocks grafted with necrotic scions, had been raised in peat and potted on in the usual way. The survivors, 139 in all, or about 25% of the grafts made, were then planted out in July 1940, being between 1–2 years' old from grafting. At this time, a single plant only showed slight indications of transmission, the stock being small and of necrotic appearance. No sections, however, were cut. The plants had not been cut back after grafting and in most of the others the stock had grown considerably larger than the scion. After planting out, the grafts were examined and recorded for possible transmission at regular intervals of 3, 4, and later of 6 months. At first, every effort was made to encourage scion growth by keeping the stocks cut back: later, in the hope of inducing transmission of the disease, a few stock shoots were allowed to develop, as far as possible in dependence on the increased vigour of the scion (see Hildebrand, 1941). By June 1942, all the remaining plants had vigorous scions in which the presence of 'phloem necrosis' was abundantly evident and yet in nine stocks only had there been any other indications of possible transmission. Further examination of these, subsequently repeated, failed to confirm these indications and in the original plant only was the transmission authenticated. In this plant, necrosis has been observed in the stock leaves regularly since January 1942, and both stock and scion are of similar diseased appearance. Up to June 1943, no further instances of transmission were recorded from the seedling grafts.

The inarches, about thirty in number, had been grafted to necrotic branches in the field and the latter afterwards separated from the parent plant as scions. In most cases the graft union was 1–2 ft. above ground level. The plants were planted out in a bed at various times between 1938 and 1940. Here again a single plant showed early indications of transmission about a year after grafting. This time the stock leaves first showed necrosis in the petiole, and a slight tendency to leaf curl was noticed later. These observations were confirmed several times during 1940, in which year the symptoms appeared to become slightly more pronounced. In 1941, however, the necrosis was again harder to find and the curl less evident, and the diagnosis of the disease has been progressively more uncertain ever since. Among the other plants a few instances of incipient transmission only were recorded, none of which has been confirmed. As with the seedling grafts, the growth of the scions was encouraged as much as possible and the stocks headed back from time to time. All the necrotic scions increased greatly in size and remained visibly diseased. The evidence for the graft transmissibility of 'phloem necrosis' furnished by the inarches and seedling grafts as such is meagre and is possibly of less significance than the evidence which they afford of the continued multiplication of the disease in the necrotic scions.

Transmission by root grafts

At the end of 1940 an attempt was made to repeat the grafting experiments using healthy material of known, i.e. clonal origin, and including 'low' as well as 'high *jât*' types. The method of root grafting was employed, grafting healthy scions on to portions of necrotic roots. The 'low *jât*' scions were obtained from the Tea Research Institute's collection of *jât* types, the mother bushes being chosen on account of their general resemblance to the type of tea grown in the district where 'phloem necrosis' is specially prevalent. The 'high *jât*' scions were from bushes in plucking on the estate or from among the recently selected clones from which it is hoped that improved planting material may eventually be distributed. In each case the scions from a single bush were kept separate, and arrangements were put in hand to multiply the type by cuttings to serve the need of future experiments.

The first lot of grafts was made in November 1940, the second in February 1941, the two lots, totalling 240 grafts, being afterwards combined. By October 1941, fifty grafts survived, of which thirty-four had made fair growth—eighteen 'low *jât*' scions and sixteen 'high *jât*'. Ten of the 'low *jât*' scions were distinctly curled in appearance and, in all, thirteen showed necrosis on sectioning part of the new leaf: on the other hand, no leaf curl or necrosis occurred among the 'high *jât*' scions. The grafts were subsequently recorded about every 3 months, being planted out in a bed in July 1942. Both in the original pots and subsequently in the ground they were given a certain amount of protection during times of drought or strong wind. The cumulative records show that up to the present time seventeen out of twenty-two 'low *jât*' scions have been recorded as necrotic, all of which show pronounced leaf curl and some a typical zizgag as well. The diagnosis is thus complete in these plants. Of the 'high *jât*' scions, three out of seventeen have had slight

necrosis affecting a few leaves only and no leaf curl or zigzag has developed in any.

These grafts adequately establish the graft transmissibility of the disease, at any rate to the 'low *jât*' material used. They also indicate a possible reason for the earlier comparative failure to infect 'high *jât*' seedlings, a question which is dealt with in the ensuing section.

Later experiments with couple grafts

By April 1942 it could be said with fair confidence that certain 'low *jât*' clones were susceptible to 'phloem necrosis' on the basis of their behaviour in the root grafts. These clones were accordingly used for a further experiment with the old seedling graft plants. By the couple-graft process, new scions were grafted both on the stock and scion branches, wherever the conditions were suitable. A total of fifty-six grafts was made, using five clones. The weather was favourable and more than 50% of the grafts appeared to have united after the first 3 months. The first symptoms of 'phloem necrosis' in the new scion growth—leaf curl, zigzag and necrosis in the midrib of the leaf—were observable by September, only 5 months from grafting. Subsequently, more grafts became infected, and the symptoms generally were intensified so that the transmission was fully confirmed. The important conclusion emerges that transmission has occurred alike from both components of the seedling grafts: of sixteen survivors, eight out of eleven 'scion' grafts and all of five 'stock' grafts are necrotic. A seedling graft with newly infected 'low *jât*' grafts on each of the original components is illustrated in Pl. 5, fig. 4. These new observations show that in the seedling grafts the original, apparently healthy, seedling stock is actually a 'carrier', showing no reaction to the virus itself, but capable of transmitting it apparently unchanged to a new, susceptible scion. Transmission from the original and still necrotic scion branch acts as a check on the validity of this conclusion and provides further confirmation of the results of the root grafts.

Summary of jât differences

In all the experiments so far described, transmission of 'phloem necrosis' has been effected with certainty to nine clones belonging to six *jâts*, one Formosan, two from Java, and three from Ceylon. *Jât* here merely indicates the source of seed from which the mother bushes were originally raised and it is the clonal progeny of individual bushes to which the results are applicable. While there appears to be a general tendency for 'low *jât*' types to be susceptible and for 'high *jâts*' to behave as 'carriers'—and the continued absence of the disease among 'high *jât*' supplies in the field is also strongly suggestive of this, or of a possible complete immunity in addition—there is no reason to suppose that individual exceptions will not occur. Many hundreds of other grafts have subsequently been made and in these and future batches the observations are being extended to include representatives of the Tea Research Institute's collection of selected, 'high *jât*' clones. It is hoped eventually to 'index' these for their reaction to 'phloem necrosis', as much as for yield and other qualities investigated hitherto.

Discussion

The question of control, and the practical significance of the 'carrying' capacity of 'high *jât*' clones, will be dealt with in a later paper. The chief problem of theoretical interest arising from those aspects so far dealt with is that of the interaction between environmental conditions and the nature of the host variety in determining the expression of symptoms. Tea is of such intense hybridity that all grades from 'carrying' to extreme susceptibility may be expected to occur under genotypic control. However, the modifying effect of the environment can alone be considered at this stage.

The effect of temperature in 'masking' the symptoms of virus diseases is well known and the altitudinal relationship of 'phloem necrosis' suggests that a similar influence is at work here, the apparent increase in severity with increasing altitude being conditioned by the decrease in average temperature. On the other hand, the difference may be accounted for merely by the relatively greater abundance of susceptible, 'low *jât*' bushes at the highest elevations, an aspect of the problem for the elucidation of which the necessary historical information is lacking. All that can be said is that the tea at 6000 ft. and higher is among the oldest in the Island and that for many years the tendency has been to plant material of increasingly 'high *jât*' character. At the highest elevations, all the evidence suggests that 'phloem necrosis' is a disease of long standing, so that the problem of its origin would appear even more difficult of solution than it is in most virus diseases. The possibility remains that its restricted distribution is merely a matter of a slow spread from the original source, or sources, but this is probably remote in view of the relatively rapid spread in the field to be discussed in a subsequent paper. A purely climatic control of the severity of the disease is further ruled out by the sporadic occurrence of severely necrotic bushes near the lower limit of the disease generally and by the results of transplant experiments where a change of altitude from 6000 to 4500 ft. has had no apparent effect on the progressive worsening of the symptoms. Unfortunately, it has not yet been possible to transplant severely affected bushes to an altitude at which 'phloem necrosis' is so far unknown. The recent work of King & Harris (1942) with 'yellow edge' of strawberries indicates that symptom expression may be

controlled as much by soil moisture as by temperature. Previous investigations generally have stressed the importance of age and vigour of growth, the usual condition being for the most vigorously growing plants to show the most pronounced symptoms of infection. The type of shoot, quite commonly encountered, which is illustrated in Pl. 4, fig. 7, is very suggestive of a connexion between climate, vigour of growth and symptoms, yet here and elsewhere the indications are that it is the regions of rapid growth which are the least severely affected. On the other hand, the general increase in severity of symptoms after pruning, and in the relatively vigorous 'tipping' shoots, is more in accord with previous observations. Extreme variation in symptoms of the type described leads naturally to the question of the possible influence of environment on the 'carrying' capacity of certain types of bush and much further work remains to be done along these lines. The whole question of the interpretation of graft transmission experiments and the diagnosis of the disease in relation to the position of the graft and the cultural treatment after grafting needs investigation along the lines of the work of Smith (1941), Hildebrand (1941–3), Bodine & Kreutzer (1942) and others. Many of the problems raised by 'phloem necrosis' in tea find a parallel among certain of the American *Prunus* virus diseases and, it may be, are raised by the viruses of woody plants in general to which, in comparison with those affecting herbaceous species, so little attention has been devoted until recently.

The nature of the 'phloem necrosis' virus necessarily remains obscure at present. Numerous early attempts at mechanical transmission were unsuccessful, although perhaps a repetition of these using clonal material of known susceptibility would be desirable. On general grounds, in view of the type of symptoms produced, easy mechanical transmissibility would not be expected and a relationship with the 'yellows' group of viruses may be forecasted. Provisionally, the 'phloem necrosis' virus can be referred to as '*Camellia* virus 1' following K. M. Smith's (1939) system, from the name of the tea plant *Camellia thea* Link. (or *C. sinensis*—see Bond, 1942). No other virus diseases of *Camellia* spp. appear to have been reported with the possible exception of the 'yellow spot' of ornamental camellias noted by Milbrath & McWhorter (1940).

Until my appointment in February 1940, these investigations were in the charge of the Mycologist at the Research Institute, Dr C. H. Gadd. Dr Gadd very kindly placed his data and experimental material entirely at my disposal, and I have endeavoured to indicate by their dates those portions of the present work for which he was chiefly responsible. His help and guidance, however, have been freely available to me throughout. Thanks are also due to Mr C. A. Loos for taking the majority of the photographs.

I am indebted to the *Tea Quarterly* for permission to reproduce certain of the Plates which have previously appeared in that Journal.

REFERENCES

Blodgett, E. C. (1943). Peach wart. *Phytopathology*, **33**, 21–32.

Bodine, E. W. & Kreutzer, W. A. (1942). Ring spot of apricot. *Phytopathology*, **32**, 179–81.

Bond, T. E. T. (1942). Studies in the vegetative growth and anatomy of the tea plant (*Camellia thea* Link.) with special reference to the phloem. I. The flush shoot. *Ann. Bot., Lond.*, n.s. **6**, 607–30.

Fulton, R. W. (1941). The behaviour of certain viruses in plant roots. *Phytopathology*, **31**, 575–98.

Gadd, C. H. (1939*a*). Disease in non-productive bushes. *Tea Quart.* **12**, 75–86.

—— (1939*b*). A virus disease of tea. *Tea Quart.* **12**, 110–30.

Garner, R. J. (1940). Methods of vegetative propagation. In *Tech. Commun. Imp. Bur. Hort. Pl. crops*, **13**, 7–22.

Hildebrand, E. M. (1941). Rapid transmission of yellow-red virosis in peach. *Contr. Boyce Thompson Inst.* **2**, 485–96.

—— (1942). Prune dwarf. *Phytopathology*, **32**, 741–51.

Hildebrand, E. M. (1943). Techniques for localising viruses and intensification of disease symptoms. Abstr. in *Phytopathology*, **33**, 18–19.

Keuchenius, A. A. M. N. (1923). Vegetative propagation of tea. *Meded. Proefst. Thee, Buitenz.*, **85**, 16 pp.

King, M. E. & Harris, R. V. (1942). Studies in strawberry virus diseases. IV. Symptom expression of yellow-edge in the variety Royal Sovereign. *J. Pomol.* **19**, 212–26.

Milbrath, J. A. & McWhorter, F. P. (1940). Camellia yellow spot—a virus disease. Abstr. in *Phytopathology*, **30**, 788.

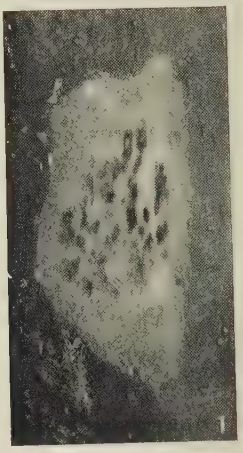
Smith, K. M. (1939). Plant viruses. II. Virus diseases of plants. *Tabul. Biol., Berl.*, **17**, 24–71.

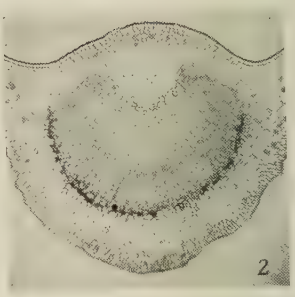
Smith, R. E. (1941). Transmission of diamond canker of the French prune. *Phytopathology*, **31**, 886–95.

Van Emden, J. H. (1940). Een nieuwe entmethode voor thee. *Arch. Theecult. Ned.-Ind.* **14**, 16–25.

Wellensiek, S. J. (1938). Een merkwaardige groeiafwijking bij thee. *Bergcultures*, **12**, 401–2.

Figs. 1–7





Figs. 1–4

EXPLANATION OF PLATES 4 AND 5

Plate 4

Fig. 1. Tea bush in fairly advanced stage of infection, rather more than a year from pruning. Dwarfing of the shoots is clearly seen. (About × 1/15.)

Fig. 2. A 'double' bush—the right-hand half severely necrotic, the left-hand healthy—apparently a natural graft union (see text). (About × 1/15.)

Fig. 3. A shoot with marked zigzag but no dwarfing. Leaves not or scarcely curled except on the main shoot below, showing a tendency to folding along the midrib. (× ¼.)

Figs. 4, 5. Typical severe symptoms affecting young shoots—dwarfing, marked curl and distortion, some zigzag. (× ¼.)

Fig. 6. Curling of the leaves only—no dwarfing or zigzag. This is the least diagnostic feature and occurs normally in healthy tea of certain *jâts*. (× ¼.)

Fig. 7. Extreme variation of symptoms in a single shoot system, probably induced by response to changing climatic or other conditions affecting the rate of growth. The centre region of the main shoot, with the much dwarfed and zigzag laterals, is typical of severely affected shoots at a rather more mature stage than that shown in figs. 4, 5. (For further explanation see text.) (× 1/6.)

Plate 5

Fig. 1. Surface view of part of the tap-root of a diseased bush, the cortex shaved away exposing severe necrotic areas of the phloem. (× 5/6.)

Fig. 2. Transverse section of midrib of diseased leaf showing the necrosis affecting the peripheral margin of the phloem. (× 28.)

Fig. 3. Illustrating grafting methods—*left*, 'couple graft'; *centre*, 'seedling graft'; *right*, 'root graft'.

Fig. 4. A 'seedling graft' top-worked with two 'couple grafts'. The original 'high *jât*' seedling was grafted in 1939 with a necrotic scion at the point marked *a*. The seedling stock is on the left, the scion on the right: three laterals have been left on each. The scion is still severely necrotic, as can be seen particularly from the dwarfed and curled shoot arising a few inches from its base. The new grafts were made in 1942 with healthy, 'low *jât*' scions at *b* and *c*. Both have become necrotic, the former by direct transmission from the original scion, the latter *via* the symptomless stock which is functioning as a 'carrier' (see discussion in text). Growing outside and removed to the pot for photographing, 8. vi. 43. (× about 1/7.)

The photographs, Pl. 4, figs. 1, 3 and 6 and Pl. 5, figs. 1 and 2, are reproduced by permission of the *Tea Quarterly*.

(*Received* 13 *July* 1943)

An unusual correlation between insect damage and crop harvested

By C. H. Gadd, D.Sc. (Birm.), *Mycologist, Tea Research Institute of Ceylon, St Coombs, Talawakelle*

An experiment is described by which an attempt was made to determine the effect of manurial treatments on the damage caused by the shot-hole borer of tea. Increases in yields of the plots were accompanied by increases in the damage caused by the beetles as measured by the number of broken branches—an unusual correlation between pest damage and harvest ($r = +0{\cdot}9267$). The experiment failed to indicate clearly the cause of increased damage following manuring, but possibly it results from an increase in the number of galleries formed ($r = +0{\cdot}6547$).

Xyleborus fornicatus Eichoff, subsp. *fornicatior* Eggers, the shot-hole borer of tea, is a Scolytid beetle which has long been regarded as one of the major pests of the tea bush in certain districts of Ceylon. The fertilized female beetle bores into living stems of suitable size, about ½ in. in diameter, and forms within the wood a characteristic gallery in which she lays her eggs and raises her family. Normally, the gallery is circular and almost completely encircles the stem; it is situated just within the woody cylinder but does not touch the cambium, and the only external evidence that a stem is attacked is the presence of small circular holes, entrances to galleries, commonly termed shot-holes. Attacked stems appear to grow normally and contribute towards the harvest of young leaf. Owing to the position and shape of the galleries, invaded stems are weakened and are liable to break at a gallery, particularly during high winds and when the bushes are being plucked. When broken the branches die and no longer contribute towards the harvest. The pest, therefore, causes a loss in crop which is difficult to estimate.

The female begins to lay eggs about 8 days after

commencing the construction of the gallery (Gadd, 1941*a*) and then continues to lay an egg daily (approx.) for a period which probably does not exceed 50 days. From the time an egg is laid until the resulting adult emerges from the gallery is about 30 days; so by the time the last of the brood takes flight a gallery will have been occupied for 80 or more days. During that time the entrance to the gallery must be kept open by the inmates to facilitate emergence, if for no other reason. But when the gallery is vacated, the entrance becomes plugged by a mass of callus tissue formed at the cambium, and the gallery is said to be 'healed'. There are therefore three classes of galleries: *occupied* galleries have inmates, and entrances are open; *empty* galleries contain no beetles at any stage of development, but the entrances are still open; *healed* galleries are empty, but the entrances are plugged with callus. It is generally held that healed galleries are less likely to break than others, but it is improbable that the mere presence of a callus plug in the entrance can account for the increased strength of the stem at such points. Healed galleries are obviously older than open galleries, and consequently, have more new wood between the gallery and the cambium, if the stem is growing at all. The new wood gives additional strength and diminishes the risk of fracture.

The view expressed by Green (1903) that 'our object should be to induce by suitable pruning and cultivation such a vigorous condition that the damage is automatically and continuously repaired' has been readily accepted by the planting community and scientists alike, and no attempt was made to test experimentally the truth of it until 1920, when Jepson started experiments to ascertain the effect of manures on a shot-hole borer population (Jepson & Gadd, 1922, 1925, 1926). The conclusions arrived at were, briefly, that no real difference occurred in the numbers of galleries found in the plots, but the percentage of healed galleries was higher in the manured than in the control plots. Actual damage resulting from the attack was not measured, but an inference was drawn that the larger percentage of healed galleries would automatically result in fewer broken stems and therefore a smaller loss in crop.

Estimation of the number of beetles in a given area at a given time bristles with difficulties. Jepson counted the galleries in selected bushes *in situ*—an arduous task and one liable to considerable error. The greatest error is likely to occur in the count of healed galleries. Jepson pointed out that 'a good deal of experience is required to decide definitely whether certain marks on the bark indicate where entrances to galleries have existed', and that often they can be disclosed only by shaving away the surface of the stem, an operation which has to be avoided as much as possible on growing bushes. A simpler method was used to follow the course of a shot-hole borer attack in a plucking experiment (Gadd, 1941*b*, 1942). Selected bushes were pruned and the prunings were removed to a laboratory where the galleries could be counted and examined under less trying conditions. The stems could be dissected and the presence of every gallery proved. The disadvantage of this method is that bushes have to be pruned at unusual times, and it cannot be used in plots from which accurate yield figures are required. Nor does it measure the actual damage caused by the beetles; the best measure of that is the number of broken branches in the plots. If the broken branches can be regarded as random samples, as the writer believes, data collected from them regarding galleries and beetles will be representative of conditions existing in the plots.

The experiment to be described was designed and laid out by Dr T. Eden to determine the effect of manurial treatments on the yield of tea in a shot-hole borer infested district of Ceylon at an elevation of 3300 ft. It consisted of four blocks, each divided into eight plots of approximately one-tenth acre in extent. The manurial treatments applied were (1) nil, (2) N, 40 lb. nitrogen/acre, (3) K, 40 lb. K_2O/acre, (4) P, 30 lb. P_2O_5/acre, (5) NK, (6) NP, (7) PK and (8) NPK. The plots were pruned in September 1940 and plucking started on 13 Dec. 1940.

The first collection of broken branches was not made until 31 Jan. 1942, when 2689 broken branches were recovered from the entire area. This collection included all branches broken since the bushes were pruned. Further collections were made weekly after each plucking, and the number from each plot was recorded. The collected branches were removed from the plots, but unfortunately, owing to pressure of other work, detailed examinations of the broken branches were not made throughout the period under review, viz. to the end of the second year from pruning. However, detailed examinations were made at the 32nd (4 Sept. 1942), 36th (2 Oct.) and the 40th (30 Oct. 1942) collections, when the numbers of each type of gallery and of the beetles at all stages of development in the occupied galleries were counted. The galleries at the break were recorded separately because it was thought probable that beetles would be lost from galleries opened at the fractures. As this proved to be the case, gallery contents were estimated from unbroken galleries only. As the number of galleries to be counted depended largely upon the number of broken branches, which varied from plot to plot, galleries have been recorded as the number in 100 broken branches, or, as in the case of healed galleries, as a percentage of galleries of all types found.

The three detailed examinations (Nos. 32, 36 and 40) were made near the end of the second year from pruning; the year-end has been taken to be 2 Oct. 1942 when the 36th examination (the middle of the three) was made. Data collected on these

occasions have been amalgamated and treated as though collected at one time, the end of the year, to represent conditions at that time (Table 1).

Discussion

Some idea of the size of broken branches may be obtained from the weight of material removed from the plots. The mean weight of 100 broken branches obtained by weighing all broken branches on six occasions (Nos. 1, 2, 17, 32, 36 and 40) was 5·639 lb. In all, 59,240 broken branches were collected from the whole area (3·2 acres) during the first 2 years from pruning, so the figures per acre become 18512·5 branches weighing 1044 lb. fresh weight. If 6000 lb. be accepted as an average weight of prunings removed from an acre of tea when unattacked bushes are normally pruned, it becomes evident that approximately 17·4% of the crop-bearing branches have been lost at some time or other during the 2 years; that loss may well reduce the crop harvested by as much as 8%. That the breakage of branches has caused some loss cannot be doubted.

As the design of the experiment is such as to allow of analysis by the method devised by Yates (1937) to determine the effects of treatment, analyses have been made to determine such effects on yield, breakages, and the number of galleries (Table 2). The results show that no main effect has emerged, though significant results were obtained for NK interactions affecting breakages and galleries. In the absence of main effects little importance can be attached to these interactions.

It is well known that tea responds readily to manurial treatment, particularly to nitrogenous manures (Eden, 1935), so it was somewhat surprising that no significant beneficial effect could be demonstrated in this experiment. The question naturally arises whether the failure can be due to a reduction in the observed effects owing to the increased damage due to shot-hole borer infestation. That increased damage has accompanied manuring is indicated by a high positive correlation ($r = +0{\cdot}92672$) existing between yield and the number of broken branches collected from the plots. An analysis of co-variance is given in Table 3, which suggests that the number of breakages varies directly with the fertility of the plots as measured by yield.

A high positive correlation between yield and insect damage is somewhat unusual. It cannot be doubted that the loss of branches from tea bushes must cause a reduction in the amount of leaf which can be harvested, yet in this experiment it is evident that those plots from which, on the average, most broken branches have been removed have nevertheless given the highest yields. There can be no question of cause and effect here; an increase in the number of breakages cannot of itself increase crop. It is more likely that the manurial treatments which tended to improve the soil fertility, and thereby increase the harvest, have also tended to increase the amount of insect damage. What has been measured as increase in yield due to treatment is not the true increase, as would occur in the absence of shot-hole borer, but only what is left after the

Table 1. *Experimental results*

			Data from three collections					
Treatment	Yield lb.	Total breakages in hundreds	Breakages	Galleries per 100 broken branches	Healed galleries per 100 broken branches	% healed galleries	Beetle contents in 100 broken branches	Mean content of occupied galleries
Nil	432·28	63·51	266	176·7	35·71	20·21	64	2·57
N	527·41	79·84	340	180·6	30·29	16·78	75	2·52
P	448·82	66·72	291	186·9	35·74	19·12	75	2·30
NP	500·20	84·08	330	200·9	47·58	23·68	91	2·97
K	540·28	86·67	399	204·8	37·59	18·36	88	2·95
NK	503·60	74·32	315	179·0	31·43	17·55	70	2·45
PK	415·56	65·15	240	190·4	31·25	16·41	84	1·88
NPK	483·37	72·11	329	181·8	34·65	19·06	67	2·92
Total	3851·52	592·40	2510	1501·1	284·24	151·17	614	—
Mean	481·44	74·05	313·75	187·6375	35·53	18·896	76·75	—

Table 2. *Effect of manures on yield and breakages and galleries*

Effect total	Yield lb.	Breakages	Galleries
N	177·64	28·30	− 82·3
P	−155·62	−16·28	46·7
NP	60·74	20·34	148·3
K	34·10	4·10	71·1
NK	−115·38	−39·08*	−235·3*
PK	−134·28	−31·18	−164·3
Significance level 5%	221·6	31·65	181·33

Significant results are indicated by an asterisk.

greater number of broken branches has decreased it. It seems probable therefore that an experiment, which may be sufficiently precise to measure differences in yields following manuring in the absence of shot-hole borer, may fail when the observed differences are smaller as a result of damage caused by that insect. We shall not concern ourselves further with that problem here.

Before considering the problem of how manuring may lead to increase in the amount of damage caused by shot-hole borer attack, it may be advisable to consider whether the observations made on the three occasions at the end of the second year from pruning are representative of the conditions that have existed during the course of the experiment. The only test that can be made is to ascertain whether the numbers of breakages on those occasions bear any relationship to the numbers collected from the plots over the whole period. The correlation coefficient for total breakages and breakages on the three occasions is +0·90496, a high and significant value, which suggests that the plots have behaved on those three occasions much as they have during the whole period, so far as breakages are concerned. It seems fair to assume therefore that other observations made at the same time are also fairly representative.

There are two obvious ways in which manuring may lead to increased damage. Some change may be brought about in the structure of the branches causing them to break more easily; then, if the infestation remains constant the breakages will become more numerous because of that change. Alternatively, the tendency of the branches to break may not be altered, but for some reason or other the branches become more attractive and more beetles make galleries; then more breakages may be expected because of the increased number of fragile places. Of course, both changes may occur simultaneously. The latter alternative will be considered first.

It has already been stated that an analysis of variance has failed to demonstrate that either nitrogen, potash or phosphate has affected the number of galleries significantly. The coefficient of correlation between the number of galleries and the breakages which occurred over the whole period has a value of +0·65466, which falls somewhat short of the significance usually demanded. Lower positive values are obtained when the number of galleries is correlated with yield, or breakages on the three occasions. There is therefore no clear indication that, by manuring, the number of galleries, i.e. the infestation, has been increased. At least, the results suggest a mere possibility.

Any change in the structure or composition of branches tending to make them more or less liable to break cannot be demonstrated directly from observations made during the course of this experiment. It is, however, generally recognized that healed galleries are less liable to break than are those with open entrances, from which the inference has been made that manuring, by promoting more rapid growth, should result in a decreased number of breakages. The data collected on three occasions can be used to demonstrate the fact that branches tend to break at open, rather than at healed galleries.

TABLE 3. *Analysis of co-variance, yields and breakages*

	D/F	S_x^2 (breaks)	S_y^2 (yield)	S_{xy}	r	Significance
Blocks	3	6·02	2897·39	−100·93	−0·76427	$P>0·1$
Treatment	7	132·15	3560·77	635·68	+0·92672	$P<0·001$
Error	21	150·30	6285·95	648·80	+0·66750	$P<0·001$
Total	31	288·47	12744·11	1183·55	+0·61728	$P<0·001$

In all, 4727 galleries, inclusive of those at the fracture, were observed and these can be classified as open or healed, and whether they were at the fracture, i.e. broken or not. The figures are set out in Table 4, from which the χ^2 value has been calculated to be 566·8, a highly significant value indicating that at the fractures there are fewer healed galleries than would be expected if both types were equally liable to cause breakage. The count of healed galleries at the break is, however, liable to error due to the fact that when a breakage occurs a plug formed in the entrance is as liable to remain on the part of the branch left on the bush as on the part carried away. The absence of the plug on examination suggests that the gallery was open and is liable to be counted as such, whereas the gallery may have been healed. Broken galleries were, therefore, counted as healed even though the plug was absent if there was any visible callus near the gallery entrance. Taking that into account it is thought that any error which may have occurred is small and not sufficient to vitiate the conclusion drawn.

TABLE 4. *Contingency table*

Expected values are given within brackets.

	Healed	Not healed		
Broken	156 (476)	2354 (2034)	2510	
Not broken	741 (265)	1476 (1952)	2217	$\chi^2=566·8$
Total	897	3830	4727	

The healing of galleries is a growth phenomenon. As manuring increases the growth rate, as shown by cropping experiments in shot-hole free areas, it has

appeared logical to assume that manuring would also speed up the healing of galleries and so decrease the breakages. That manuring has not decreased breakages is clearly shown by the foregoing, so it will not be surprising that no beneficial effect of manuring on healing could be demonstrated by analysis of variance. A few correlation ratios involving healed galleries are shown, amongst others, in Table 5, from which it will be seen that the highest value was obtained for the correlation between the numbers of galleries and the numbers of healed

TABLE 5. *Correlation ratios*

Characters	Ratio
Yield:	
and all breakages	+0·9267
and no. galleries	+0·3388
All breakages:	
and breaks on three occasions	+0·9050
and no. galleries	+0·6547
and no. healed galleries	+0·4525
and % healed galleries	+0·2603
Breaks on three occasions:	
and no. galleries	+0·4867
and no. healed galleries	+0·2746
and % healed galleries	+0·1231
and beetle contents of galleries	+0·3105
No. of galleries and no. of healed galleries	+0·6445
Level of significance $P=0·05$	0·7067

galleries, viz. $r=+0·64454$, which falls short of significance but tends to indicate that the number of healed galleries increases as the number of galleries in general increase. That suggests that the rate of healing in all plots has been fairly uniform, i.e. manuring has not increased the rate of healing.

The results of this experiment therefore do not support the current view that the maintenance of a vigorous condition in the tea bush by manuring will diminish the damage caused by shot-hole borer by enabling the bush automatically and continuously to strengthen branches weakened by attack. On the contrary, the evidence indicates that damage is increased, possibly though not certainly, owing to an increase in the number of galleries formed in the stems.

In the last column of Table 1 are given the mean numbers of beetles found in occupied galleries from plots of each treatment. They range from 1·88 to 2·97, and as these values include female parents, one in each gallery, the number of beetles in young stages is obviously small. That suggests a cessation or a diminution in the rate of breeding. This was not altogether unexpected, as a previous experiment (Gadd, 1942) indicated that the numbers of new galleries formed in the stems diminished towards the end of the second year and throughout the third year from pruning. The numbers of broken branches in this experiment indicate that the attack has taken a similar course. In February 1942 the mean number of broken branches after each plucking was 1205; by June the number had risen to 2450, after which the numbers decreased to 1162 in September. In the third year from pruning the number of broken branches has continued to decrease, and in February 1943 the mean number per collection was only 268. The observations are of interest not only because they indicate the probability of a change of some sort in the stems which makes them less attractive or less suitable for breeding in during the third year from pruning, but because they suggest the possibility that with much diminished damage in the third year the manures will then have a significant effect on yields. The cropping figures during the third year will therefore be of particular interest.

ACKNOWLEDGEMENTS

My thanks are tendered to Dr T. Eden for permission to use his experiment for the collection of data used here and for his helpful advice on the use of statistical methods; and to G. D. Austin and W. T. Fonseka who collected the field data.

REFERENCES

EDEN, T. (1935). Studies in the yield of tea. Part III. Field experiments with potash and nitrogen in relation to the pruning cycle. *Emp. J. exp. Agric.* **3**, 105.

GADD, C. H. (1941*a*). The life history of the shot-hole borer of tea. *Tea Quart.* **14**, 5.

—— (1941*b*). Observations on an attack by shot-hole borer on tea. *Tea Quart.* **14**, 132.

—— (1942). The effect of plucking on shot-hole borer attack in tea. *Tea Quart.* **15**, 31.

GREEN, E. E. (1903). Shot-hole borer. *Circ. agric. J. Ceylon*, **2**, no. 9, 153.

JEPSON, F. P. & GADD, C. H. (1922). The effects of manures on the shot-hole borer of tea. *Bull. Dep. Agric. Ceylon*, no. 56.

—— —— (1925). The control of shot-hole borer of tea. *Bull. Dep. Agric. Ceylon*, no. 72.

—— —— (1926). Manuring in relation to the control of shot-hole borer of tea. *Bull. Dep. Agric. Ceylon*, no. 78.

YATES, F. (1937). The design and analysis of factorial experiments. *Tech. Commun. Imp. Bur. Soil Sci.* no. 35.

(*Received* 9 *September* 1943)

Studies of wireworm populations

I. A census of wireworms in pasture

By George Salt, Sc.D. (*King's College*) and F. S. J. Hollick, Ph.D. (*St John's College*)
From the Zoological Laboratory, Cambridge

(With 7 Text-figures)

A method has been developed, and is here briefly described, by means of which all the wireworm larvae of all stages can be collected from soil samples. By use of that method, complete wireworm populations have been obtained from soil samples of three types, two of which provide large homogeneous populations for detailed study while the third gives information about the seasonal and spatial infestation of fields. These collections show that the wireworm population of English pasture land is on the average about three times as large as has been commonly supposed. Populations have been found ranging up to ten millions per acre in the top 12 in. of soil. In two pastures studied intensively throughout the year, the wireworm population has been found to consist of large numbers of small larvae, decreasing numbers of larger larvae, and comparatively few of the very large larvae that have usually been allowed to represent the population. Such a composition is shown to be characteristic of wireworm populations under old grass at all seasons of the year and in several fields in different parts of the country. This result suggests that in research on the wireworm problem it is desirable to take into consideration the whole wireworm population throughout the year, not merely the large larvae during the cropping season.

General introduction to the series

It is now generally accepted, and needs no argument, that the efficient practice of economic entomology requires quantitative information. The problems of economic entomology originate in the excessive abundance of an insect and in the amount of damage it does. Their solutions depend on the numbers of the pest destroyed and the proportion of damage averted, compared with the amount of labour or the cost involved in the controlling operation. In short, numbers and quantities lie at the root of all studies directed towards the control of an insect pest. The general recognition of this fact is sufficiently attested by the large number of papers published in recent volumes of this *Journal* on the subject of insect populations.

In the case of the wireworm problem, the matter of numbers is especially important, for two reasons. The first is that the official method of dealing with the pest at present is to avoid it. Of two pastures, that is ploughed where there are fewer wireworms to attack the subsequent crop. Of two crops, the more susceptible to damage is planted in the field where there are fewer wireworms. The method depends upon a knowledge of the number of wireworms present. The second reason is that wireworms are unusual among insect pests in having a life cycle extending over a period of years. Their menace to a particular crop is a function of the number of small larvae hatched 3 or 4 years previously and of the environmental conditions that have occurred in the intervening period. Some of those conditions, it is to be noted, may not have affected the whole population but only that part of it old enough to have experienced them. For instance, the older larvae may have endured a hard winter or a severe drought to which the younger larvae were not exposed. To trace the environmental factors that have most influenced a wireworm infestation therefore involves a knowledge, not merely of the gross number of wireworms that survive, but also of the composition of the population with regard to the number of individuals of different ages.

In spite of the importance of numbers in the study of wireworms, little or nothing is known about complete wireworm populations in nature. There are many papers, of course, in which the number of wireworm larvae per acre or per cubic foot of soil is stated with more or less assurance, but it is doubtful whether any of these published numbers refer to complete populations. It is often difficult, if not impossible, to discover what they do refer to. Those of which we have been able to obtain information have proved to represent only partial populations comprising an uncertain and varying proportion of the larger larvae and only very few of the smaller ones.

It may be useful in agricultural practice to count the large wireworms, since they appear to do most of the damage and therefore require immediate control; but for the ultimate solution of the wireworm problem the smaller larvae are just as important as the large, and a knowledge of the complete population is essential. For instance, a treatment which appears to have no effect on the older larvae may be

very effective against the younger stages, and so ensure a control of the pest in 2 or 3 years' time. Or again, the distribution of wireworms in relation to edaphic and climatic factors (a subject now in great confusion) cannot be explained with reference only to the hardy older larvae, but must take into account the effect of those factors on the delicate early stages.

This is the point of view which has actuated us, two academic biologists, in contributing our spare time to the war effort by research on the wireworm problem. Holding that fundamental research on wireworms involved a knowledge of complete populations, we directed our first efforts to the development of a reliable method for collecting from soil samples all the wireworm larvae of all stages. That method was made known to Advisory Entomologists and others by means of a mimeographed report circulated in March 1941. Next we applied our method to the collection of a complete wireworm population from a particular piece of turf at a particular time; and a study of that collection enabled us to give, in the same report, what we believe to be the first description of a complete wireworm population. Extending our observations in time, by regular collections throughout the year, and in space, by making our collections at definite stations in particular fields, we were able in September 1942 to circulate a second report dealing in a preliminary fashion with the seasonal development of a wireworm population and with the distribution of wireworms within fields. All this work was confined to grassland, because it seems to us evident that a knowledge of wireworms in their natural habitat, under grass, is fundamental to an understanding of their occurrence in the artificial conditions of an arable field. Having established that knowledge, we began our study of the changes that take place in the population when grassland is ploughed, and have also made preliminary observations of the effects of different methods of cultivation.

This outline shows clearly enough the direction in which we have carried our work. The publication of our results is expected to follow a similar course, except as regards the description of our method of extracting wireworms from soil samples. In this paper we give only a brief description of the method in the form in which we have used it in this research. An account of its development and subsequent modification for large-scale work will be published later. The principal purpose of the present paper is to describe a census of wireworms from grassland both as to number and as to the composition of the population.

SOURCE AND TYPES OF SAMPLES

The natural populations to be described occur principally in two fields. The first, Sheep's Green, was used because it is near our laboratory. It is a low-lying common, of about 7 acres, intersected by small streams and ditches, and sparsely planted with willows and Lombardy poplars. Its soil is a shallow clay loam with heavy clay beneath. Our work in this field has been concentrated in one small part, comprising about ¼ acre; free of trees, although bordered by them; and covered principally with the grasses *Hordeum pratense*, *Agrostis alba*, *A. tenuis* and *Phleum pratense*, with clumps of *Dactylis glomerata* and small cushions of *Festuca ovina*; and with *Ranunculus repens*, *R. acris*, *Cnicus arvensis* and *Crepis virens* as the principal weeds. For the determination of these and other plant species mentioned in this paper we are grateful to H. Gilbert-Carter, M.A., of the University Botanic Garden. It is unlikely that Sheep's Green was ever under cultivation. During the last few years it has been only lightly grazed and rarely, if ever, cut for hay.

The second field, Spinney Pasture, is an ordinary pasture of 8 acres, on the University Farm, some two miles north-west of Cambridge. It lies on a slight eastern slope, is ditched along the north and east, and has a small spinney of larch, fir and hornbeam on the west. The pasture and the spinney are surrounded by arable land. The soil is a very heavy clay (gault). This field has been continuously under grass, used for pasture and cut for hay, for at least 10 years. Its principal flora consists of the grasses *Agrostis stolonifera*, *Lolium perenne*, *Trisetum flavescens* and *Hordeum pratense*, with tufts of *Dactylis glomerata* and small patches of *Festuca ovina* and with some scattered *Phleum pratense*. There is a good deal of clover (*Trifolium repens*) and, in parts of the field, of the moss *Brachythecium rutabulum*. The most prominent weed is *Cnicus arvensis*, but there are also scattered plants of *Cnicus lanceolatus*, *Taraxacum*, *Bellis*, *Potentilla*, *Veronica*, etc. We should like to record our gratitude to W. S. Mansfield, M.A., Director of the University Farm, for making it possible for us to study this field so intensively.

From these two fields, three types of samples have been taken: square-foot samples, square-yard samples, and the more usual, cylindrical, 4 in. samples. The first comprise the soil from an area 1 ft. square removed to a depth of 18 in. in twenty-seven separate parts each 4 in. square and 6 in. deep. These samples, which were taken only from Sheep's Green, serve to provide the large numbers of wireworms collected at the same time and in the same place which are necessary for population analysis. Since one or two of them were taken at the beginning of each month from March 1941 to April 1942, they also give information on seasonal changes in the composition of wireworm populations. But, because only one or two such samples could be examined each month, and because most of them were taken from a part of Sheep's Green known to be heavily infested, they give no reliable information on the

changes that take place in the course of a year in the total number of wireworms, and they do not represent the infestation of the field as a whole.

Square-yard samples were taken with a view to studies of the micro-distribution of wireworms. They include all the soil from an area 1 yd. square removed to a depth of 6 in. in eighty-one separate parts each 4 in. square and 6 in. deep. As well as giving information on the distribution of wireworms with reference to the plant cover and root systems, these samples provide especially large homogeneous populations for analysis.

The 4 in. samples were taken with the ordinary boring tool which removes a cylinder of soil 4 in. in diameter and 6 in. deep. In our work, we have invariably taken two such cores at each station, the upper from the surface to a depth of 6 in., the lower from 6 in. to 12 in. deep, and have examined them separately so as to learn how many wireworms were above and how many below a depth of 6 in. We have used this type of sample to provide information on the distribution of wireworms within fields and, for that reason, we have not taken them at random but at definite stations and at regular monthly intervals. At Sheep's Green, sixteen stations were ranged in two rows across the field and samples were taken near them at the middle of each month from July 1941 to September 1942. At Spinney Pasture, twenty stations, arranged in five rows across the field, were sampled in the last week of each month from December 1941 to the present.

All the different kinds of soil samples described above were immediately placed, in the field, into tins with tightly fitting lids. Tins are less convenient to handle than bags; but they have the advantages that all elements of the fauna are confined and that the soil retains its natural moisture. Before taking a sample, we cut off the growing vegetation close to the surface of the ground with scissors, so as to avoid including unnecessary and complicating material.

Method of examining soil samples

The technical problem to be solved in collecting the complete wireworm population from a soil sample can be conveniently considered in four parts: (1) breaking the lumps of soil so as to release any wireworms they may contain; (2) freeing the wireworms from all vegetation in which they may have become entangled; (3) removing the wireworms (and other like material) from the soil; (4) separating the wireworms specifically from all other material, animal or vegetable, collected with them.

(1) *Breaking the lumps of soil*

Preliminary experiments showed that small wireworms could be recovered from sieved lumps of soil as small as 5 mm. in diameter. Lumps much smaller than this are not likely to imprison larvae, because the presence of a larva in a lump weakens its resistance to breakage; but, in order to be certain of releasing all larvae, we decided to break up all lumps of soil to a diameter of 2·5 mm. or less.

The method finally adopted for this purpose depends on freezing. Each part of the sample is brought from the field in a small tin box, $7\frac{1}{2} \times 6 \times 6$ in. On arrival at the laboratory, water is poured into the tin, enough to cover the soil, and the sample is put at a temperature of $-12°$ C. After freezing for 48 hr., or as much longer as is convenient, the tin is put at room temperature and in about 24 hr. the sample has thawed and is ready for further treatment.

The soil is now washed under jets of water through two wire sieves (Fig. 1, *a* and *b*) into a reservoir (Fig. 1, *c*). The three vessels are arranged on a stand

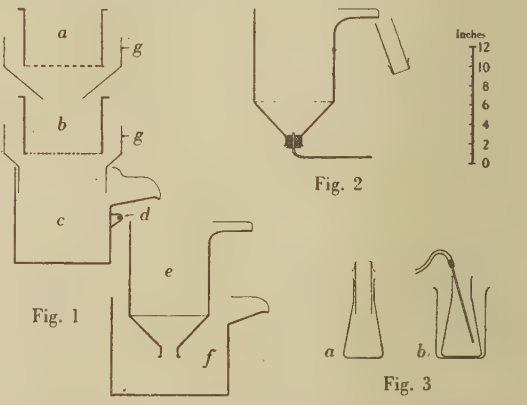


Fig. 1

Fig. 2

Fig. 3

Fig. 1. Arrangement of apparatus for sieving. *a*, first sieve; *b*, second sieve; *c*, reservoir; *d*, pivot about which reservoir is tilted; *e*, flotation vessel; *f*, tank; *g*, *g*, splash guards.

Fig. 2. Arrangement of apparatus for flotation.

Fig. 3. Apparatus used for separation of wireworms. *a*, flask receiving material from lamp-glass; *b*, arrangement for overflow of material from the benzene layer.

fitted with sloping guards to catch and return any splashes. The two sieves have square meshes, 7 and 2·5 mm. across, respectively. Even heavy clay soils are so disintegrated by the freezing that, under the action of the water, they readily pass through both sieves. In short, the soil has been broken up into particles less than 2·5 mm. in diameter; too small, that is, to entrap a wireworm larva. The first object is thus attained.

(2) *Freeing the larvae from vegetation*

When all the soil has been washed through the two sieves, there remain long roots and large pieces of vegetable matter retained by the 7 mm. sieve, and shorter roots, small mollusc shells, and other debris in the 2·5 mm. sieve. The long roots in the uppermost sieve are now pulled apart and washed under

so strong a jet of water that no larva could remain entangled by them. Then, after a rapid examination, they are discarded.

The vegetable and other debris in the 2·5 mm. sieve is first washed thoroughly and, if necessary, is pulled apart, although most of it is too short to be tangled. Much of it is driven through the sieve, and that which is still retained after thorough washing is added to the material accumulated in the reservoir (Fig. 1, *c*) by inverting the sieve and washing out its contents. The whole apparatus is now washed down so that, with the exception of the longest roots discarded from the uppermost sieve, the whole sample is brought together in a finely divided state in the reservoir.

(3) *Removing the wireworms from the soil*

In the meantime, overflow water from the reservoir (Fig. 1, *c*), and some of the lighter material carried by it, has been flowing from the reservoir by means of a deeply cut lip into a fourth vessel of special design (Fig. 1, *e* and Fig. 2). Built into this fourth vessel is the third and finest sieve, of copper wire, with a square mesh, 0·2 mm. across. This mesh is fine enough to retain all stages of the wireworm, even the eggs or newly-hatched larvae, but it allows very fine silt to escape.

The reservoir is now tilted about its supporting pivot (Fig. 1, *d*) and the whole contents are thus poured into the fourth vessel. A jet of water, keeping the soil in suspension and the sieve unobstructed, gradually washes the silt through the fine sieve and away into silt traps leading to a drain. Obstruction of the sieve is also avoided by its immersion in water, in a tank (Fig. 1, *f*) which maintains the water inside and outside the vessel at a level just above that of the sieve.

The fourth vessel now contains the whole sample, except the long roots and the silt, in a finely divided and washed condition. The water jets are now turned off; the fourth vessel is lifted; and the water in it is drained through the sieve. The vessel is then transferred to a stand. The sides of this vessel below the sieve form a funnel, the mouth of which is now closed by a rubber bung carrying a jet of compressed air (Fig. 2). The air jet is turned on, and then a solution of magnesium sulphate having a specific gravity of about 1·2 is poured into the vessel. The air bubbles up through the whole surface of the sieve into the magnesium sulphate, keeping the soil particles in suspension and freeing all the organic matter that will float in a solution of such specific gravity. The bubbling is stopped by turning off the jet of compressed air; the soil settles; the level of the liquid is raised by adding more of the solution slowly, through a delivery tube opening under the surface; and the floating organic material, including the wireworms, passes over a carefully designed lip to fall into a lamp-glass. The lower end of the lamp-glass is covered by a piece of bolting-silk held in place by means of an elastic band. The bolting-silk retains the material and allows the recovery of clean solution. A second period of bubbling, stirring and settling ensures that nothing having a specific gravity less than 1·2 is trapped by the soil. Any further material that rises to the surface is collected with the first. The magnesium sulphate is now decanted from the vessel to be concentrated and used again, and the soil, from which all floatable material has been removed, is at last discarded. In its use of air bubbling through a solution of magnesium sulphate, this part of our method is indebted to the late W. R. S. Ladell (1936).

(4) *Separating wireworms from other organic material*

The mass of vegetation and other organic material collected in the bolting-silk sieve is first thoroughly washed, and then put into a wide-necked flask with a small quantity of water (Fig. 3, *a*). Benzene is added and the whole is violently shaken. Since the insect cuticle is wetted by benzene while most vegetable material is not, the wireworms and other insect material accumulate on the water-benzene interface, where they can be seen lying in the benzene above the vegetable matter floating in the water beneath. In order to throw down as much vegetable matter as possible, the pressure within the flask is now reduced with a suction pump. This at first causes much of the material that has sunk in the water to rise to the interface; but when atmospheric pressure is restored much of the vegetation, robbed of the air which formerly made it float, now sinks.

By means of a slow jet of water, introduced below the interface, the benzene and all floating material is overflowed into a large beaker, leaving the non-floating vegetation in the flask (Fig. 3, *b*). The flask is washed down and removed, and in the beaker the wireworm larvae now stand out with surprising clarity on the interface and are easily collected with a brush or a pair of forceps. A jet of air which disturbs the benzene and makes the insect material move over the undisturbed interface is a considerable aid to the eye. When the wireworms have been collected, most of the benzene can be recovered.

Since the object of our method is to obtain all the wireworms of all stages, exhaustive tests have been made at each step of the process to discover whether any are lost. Larvae so severely damaged that a large part of their viscera is uncovered by cuticle may be less firmly supported in the benzene; but this or any other loss, if it occurs, must be very small because third periods of bubbling and flotation, second treatments with benzene, and thorough examinations of residues about to be discarded have seldom recovered an additional larva, and never more than one in any sample. We therefore proceed on the assumption, which we have no reason to doubt, that

our collections include so high a proportion (we think, over 99%) of the wireworms present in the soil samples that, within the range of significance, we can consider them as complete populations.

An individual worker using this method can deal with a standard sample of difficult clay soil in about 1½ hr. This rate is sufficient for research purposes, but too slow for large-scale surveys. To provide for this, we have recently made modifications which so accelerate the method as to make it fully suitable for the national wireworm survey.

Material obtained from the samples

Our method of examining soil samples, described above, has been developed in response to the particular needs of the wireworm problem, but, of course, it yields many elements of the soil fauna other than wireworms. Larvae and adults of other beetles, fly maggots, ants, mites, eel-worm cysts and animals of other groups are recovered by the same process, provided only that their cuticle has the property of being wetted by benzene, and that they are light enough to be buoyed up by the film of benzene about them.

In the present research we have not regularly collected insect material other than Elateridae, although we have noted the presence of other groups, principally predaceous in habit, which we thought might affect the population of wireworms. Among the Elateridae we have principally been concerned with species of *Agriotes* and, although we have collected considerable material of and information about *Athous*, it is of *Agriotes* alone that we write in this paper.

Of the three species of *Agriotes* of economic importance in England, one, *A. sputator*, is predominant in both of the fields we have studied intensively. At Sheep's Green it appears that about 95% of the *Agriotes* population belongs to that species, since 2211 of the 2339 larvae and adults we have determined from that field were *sputator*. At Spinney Pasture the wireworm population is almost exclusively *sputator*—of 2629 adults and larvae that have been named, 2603, or just over 99%, were that species.

All stages of *Agriotes* can be recovered by our method of examining soil samples, but we have not held ourselves responsible for actually collecting all the eggs. We have merely noted their presence and approximate number in each sample, and preserved some specimens for identification. In many cases the eggs contained larvae so far developed that they could be generically identified by the pits on the ninth abdominal segment. Apart from the eggs, we have zealously sought and kept all stages of the insect. Our very large collections of larvae, to be described below, include a great number less than 3 mm. long, and give confidence that our method extracts even the smallest larvae efficiently. Pupae are sometimes broken by the jets of water in the sieves, but the separate pieces collect on the benzene interface and are so few that they can easily be associated for accurate counting. Adults are recovered in such excellent condition that they can be dissected, the development of their gonads examined, and the number of eggs they contain counted.

Size of the wireworm population

From each of the sixteen stations at Sheep's Green, we have examined eighteen 4 in. samples. The 288 samples contained 1956 larvae of *Agriotes*, an average of 6·8 per sample. This figure represents a population of about 3,400,000 wireworms per acre. From each of the twenty stations at Spinney Pasture, we have examined one sample in each month during 1942. The 240 samples contained 3742 larvae of *Agriotes*, an average of 15·6 per sample, representing a wireworm infestation of 7,800,000 per acre.

Taken by themselves, these figures are of little interest. Considered in relation to published figures of wireworm infestation, such as those of Roebuck (1924), Miles & Cohen (1938), and Jary (1942), they show that the two fields have very high wireworm populations, but that is of no general importance. It is only when the number of wireworms recovered by our method is directly compared with the number recovered by ordinary methods of sampling that our figures begin to show their real significance.

The direct comparison is possible because during the winter of 1941–2, at the request of Mr J. C. F. Fryer and with the helpful co-operation of the Advisory Entomologists concerned, we were able to sample ten fields in comparison with the Ministry of Agriculture advisory teams. We are grateful to Messrs Fryer, Jary and Petherbridge and to Dr Miles both for making the comparisons possible and for allowing reference to the results. It is to be understood that the hand-sorting methods used by the advisory teams were not designed for research but for advisory purposes. As applied in the wireworm survey, those methods have given counts sufficiently accurate to show correlation with crop yields (Finney, 1941); but it is not surprising that they should be found to yield fewer wireworms than a research method designed for the study of complete populations.

In making these comparisons, the two teams took adjacent and similar cores at twenty places in each field, and each team examined the soil in its customary manner. The results of the comparison are summarized in Table 1.

From these ten fields, advisory sampling methods recovered 38% of the number of wireworms obtained by our method from comparable upper cores. But we found an additional number of wireworms, amounting to 14% of our total collection, in the

lower cores, not normally taken in advisory work. The advisory collections therefore represent less than 33% of the number of wireworms present in the upper 12 in. of soil.

There is further evidence to support this estimate. On two occasions, when the advisory team had completed its examination of soil samples and had removed the wireworms they had found, the soil was put aside and examined again by our method. For the first instance, we are indebted to Mr Petherbridge. Ten samples from the East Anglian province, which had yielded the advisory team thirty-five wireworms, were found to contain sixty-six more when subsequently examined by us. In this case, then, the advisory team found just under 35% of the total number of wireworms present. In the second instance, Mr Staniland kindly sent us from among the few fields for which we have presented data in Table 1, one has a wireworm population exceeding ten millions per acre in the top 12 in. of soil. But to find the heaviest infestation of wireworms has been no part of our object. In our view, the collection of a complete wireworm population is less interesting for the high figure it gives of the number of wireworms present than for the information it provides about the composition of wireworm populations.

Composition of the wireworm population

Since the larval stage of *Agriotes* extends over a period of about 4 years, while the other stages are relatively short-lived, by far the greatest part of a complete population of *Agriotes* consists of larvae.

TABLE 1. *Comparison of the number of* Agriotes *found by advisory methods and by Salt & Hollick*

Field	District	Soil	Number of *Agriotes* found in upper core by: Advisory team	Number of *Agriotes* found in upper core by: Salt & Hollick	Additional *Agriotes* found by Salt & Hollick in lower core
1	Cambridge	Clay	55	229	34
2	,,	,,	48	293	41
3	Manchester	Sandy peat	3	41	6
4	Norfolk	Black fen	17	21	*
5	Manchester	Loamy sand	19	56	7
6	,,	Heavy loam	0	6	0
7	Norfolk	Black fen	74	116	*
8	Cambridge	Clay	169	246	51
9	Romney Marsh	Silty loam	120	337	67
10	,,	,,	74	187	21
Total	—	—	579	1532	227

* In these two fields both teams sampled to a depth of 12 in., and the collection from both the upper and the lower cores is included in the other columns.

Devon the soil of twenty-six samples from which his collectors had removed fifty-one larvae of *Agriotes*. In the soil we found a further ninety. The advisory team in this case found fifty-one of the 141 wireworms present, or 36·2%. But both of these cases refer only to the upper 6 in. of soil. If an allowance is made for 14% of the population living below 6 in. deep in these two fields, as in the other ten, then the numbers recovered by the advisory teams in the two cases represent 30 and 31% respectively of the true population. It should be borne in mind that it is not the prowess of the teams but the number of wireworms recovered by different methods that is here being compared and that, as already stated, the advisory methods and our own were designed for different purposes.

So far, then, as these opportunities for comparison may represent the normal state of affairs, it would appear that, on the average, the true wireworm population of English fields is about three times larger than published figures might suggest. Even Among those larvae, there are present in any complete wireworm population, individuals which are in their first, second, third or fourth years, and which range in length from about 3 to about 20 mm. As a first step in the analysis of wireworm populations, we have measured several thousands of larvae recovered from our various samples.

Measuring larvae of this type is difficult because they tend to lie in a curved position, and much more because the degree of extension or retraction of their intersegmental membranes enormously affects their apparent length. We have measured all our larvae after preservation in 70% alcohol. We first extend them fully by gently pulling or squeezing them with forceps and we measure them when at maximum extension by holding them quite straight and under slight pressure between the tines of curved forceps. This treatment provides measurements which are about 1·2 times the length in an average living position. The measurements have all been made with a squared eye-piece micrometer in a binocular

dissecting microscope having such a lens combination that the field is divided into squares of 0·4 mm. side. By this means, the larvae have been separated into size groups 5·1 to 6, 6·1 to 7, 7·1 to 8, and so on, units long, where each unit is 0·4 mm.

Let us first consider the composition of the wireworm population of Sheep's Green as revealed by our several types of samples. From 288 4 in. samples we recovered 1956 wireworm larvae; from seventeen square-foot samples, taken principally from a part of the field known to be heavily infested, we collected 3070 larvae; from two square-yard samples we obtained 1481 larvae. We have, then, a total of 6507 larvae from this one field. All of them have been measured, and the measurements are presented by means of the histogram (Fig. 4).

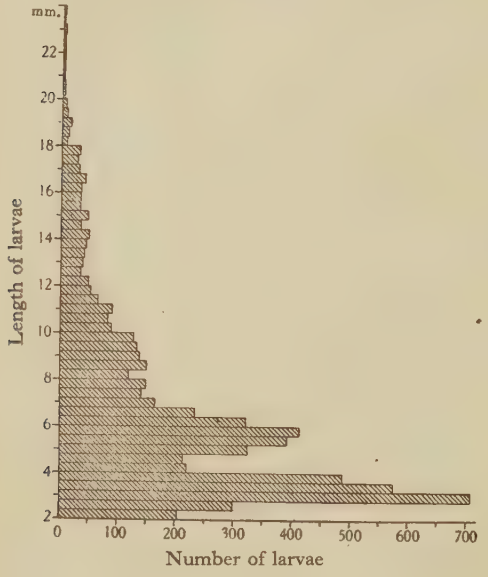


Fig. 4. Summary of measurements of 6507 larvae of *Agriotes* from Sheep's Green.

One glance at the figure shows the principal result of this first step in the analysis of a wireworm population. This large collection of wireworms from old grassland is mainly composed of small larvae. About three-quarters (74·4%) of the total collection are larvae less than 8 mm. in length.

Not only are the smaller larvae in the majority, but there is also, in general, a very marked correlation between the length of the larvae and their numbers—there are many larvae in the smaller size-groups, and progressively decreasing numbers of the larger sizes. Nearly 59% of the whole collection falls into the ten size-groups between 2 and 6 mm.; another 26% between 6·1 and 10 mm.; only 9% between 10·1 and 14 mm.; and only 6% in the twenty-five size-groups between 14·1 and 24 mm.

It should be observed in passing that even in this agglomerate collection of wireworms, the numbers in the different size-groups do not decrease quite steadily. There are fewer larvae in the two size-groups between 4·1 and 4·8 mm. than there are in the size-groups about 6 mm., so that the shape of the histogram is abruptly narrowed at this point. A similar constriction is noticeable in the region of the 8 mm. group, and another, less strongly marked, occurs among the higher size-groups. In our report to the Advisory Entomologists dated March 1941, we suggested that the constrictions in such a histogram mark off the larvae in the first year of their larval life from those in their second year, and so on; and therefore that the larger size-groups they delimit represent year-groups. In such a collection as that represented in Fig. 4, a collection made at intervals throughout the year, any grouping apt to be revealed by measurements of length would naturally be obscured by growth. It is only when a homogeneous population, sampled at one time, is considered alone, that the separation of the collection into a few large size-groups is shown most clearly. But we must not anticipate work to be described later. Here we wish merely to call attention to the possibility that the natural size-groups apparent in the histogram may represent age-groups.

Before the composition of this collection of wireworm larvae can be accepted as having any general validity, two further sets of data are necessary. It must be shown, namely, that it does not represent the composition of the wireworm population only at particular seasons of the year; and that it is not peculiar to the particular field, Sheep's Green, from which it was taken.

The possibility of seasonal bias can readily be cleared away, we have taken at least one square-foot sample and one set of 4 in. samples from Sheep's Green in every month of the year. For each month, therefore, we have a collection of at least 200–300 larvae. Without exception, each of these monthly collections is composed of a high proportion of small larvae and diminishing numbers of the larger ones. It is unnecessary to consider these monthly collections in detail here. The point we wish to make, that the population structure shown in Fig. 4 is not peculiar to a particular season or seasons, is sufficiently illustrated by the histograms for four three-monthly periods of the year, shown in Fig. 5. In drawing these histograms, since we had collected unequal numbers of larvae in different quarters of the year, we have not plotted the absolute number of larvae in each size-group. Instead, we have calculated as a percentage the proportion of each size-group in each monthly collection, and have added the monthly percentages for each quarter of the year. This provides four histograms of the same area. These show without any doubt that in each season of the year, winter, spring, summer, and

autumn, the wireworm population is composed essentially as we have described it, of large numbers of small larvae and decreasing numbers of the larger ones.

To show that the composition of the wireworm population of Sheep's Green is not a peculiarity of that particular field, let us first consider the collections from Spinney Pasture. These collections include the wireworms from 276 4 in. samples, sixteen taken in October 1941 and twenty in each month from December 1941 to the end of 1942. The 276 samples yielded a total of 4266 larvae. A square-yard sample taken from Spinney Pasture in August provided a further 1334 larvae. Measured and recorded as those from Sheep's Green, these 5600 larvae form the histogram of Fig. 6, and show that, in the preponderance of small larvae, the composition of the wireworm population of Spinney Pasture is essentially similar to that of Sheep's Green. In this case 52% of the collection falls into the ten size-groups between 2 and 6 mm.; 23% between 6·1 and 10 mm.; 12% between 10·1 and 14 mm.; 7% between 14·1 and 18 mm.; and 6% over 18·1 mm. in length. The principal difference between the two fields, the fewer

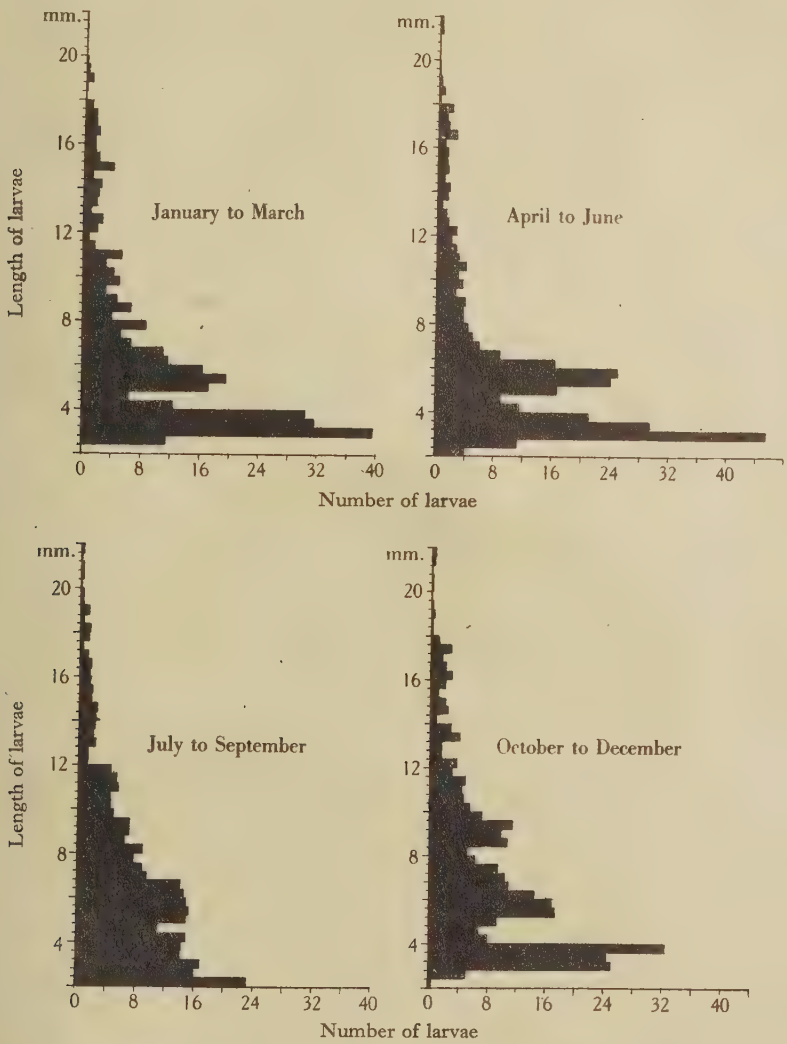


Fig. 5. The seasonal composition of the *Agriotes* population of Sheep's Green.

larvae in Spinney Pasture of a length about 7 to 8 mm., can be accounted for by the history of the population.

We have sampled five other pastures in Cambridgeshire and in each of them we have found that the wireworm population has a composition similar to that of Sheep's Green. From one of those fields, at Boxworth, we have a collection of 548 larvae; from another, near Lolworth, 179; from a small field at Papworth St Agnes we have 242 larvae; and from two large adjoining fields near Swavesey 462 and 511. The composition of these five collections is summarized in the first five histograms of Fig. 7. In their general composition, with which we are here

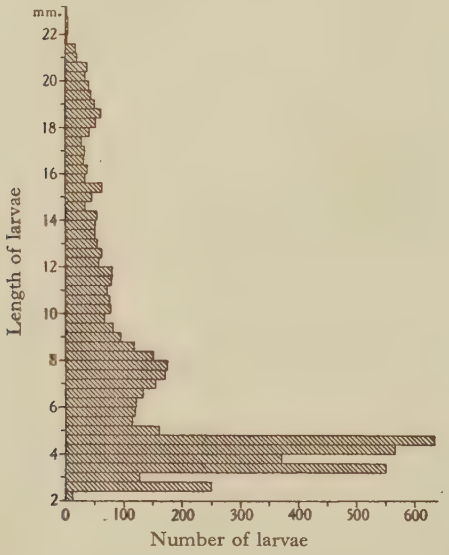


Fig. 6. Summary of measurements of 5600 larvae of *Agriotes* from Spinney Pasture.

alone concerned, these five collections agree closely with the much larger collections we have described from Sheep's Green and Spinney Pasture.

That the composition of the wireworm populations found in Cambridgeshire is not peculiar to the pastures of this county, is indicated by the collections from a few fields we have sampled in other parts of England. As recorded above (p. 56) we enjoyed the co-operation of the Advisory Entomologists in taking samples from three different advisory provinces. Because these distant fields were each sampled only once, the number of larvae collected from them was comparatively small; but from two adjoining fields on Romney Marsh, Kent, we obtained a total of 608 larvae; from three fields widely separated but all in the neighbourhood of Manchester, 113 larvae; and from two fields in Norfolk, 136 larvae. The composition of these three collections is illustrated in the last three histograms of Fig. 7.

It can be seen at a glance that the first two collections, from Romney Marsh and Manchester, take the now familiar form. The Norfolk collection, however, differs very noticeably in lacking the large number of larvae of the smallest size-groups, which are so constant a feature of our other collections. This peculiarity is entirely due to one field (number 7 in Table 1), since the small collection from the other Norfolk field, for what it is worth, has the usual composition. Now it is to be observed that, whereas all the other fields we have described were under a normal close cover of grass, this field of black fen soil was very sparsely covered with vegetation. In his report upon it, Mr J. Stapley, the Assistant Advisory Entomologist who sampled with us, estimated that between one-quarter and one-third of the area of the field was exposed as bare soil, and recorded that most of the remaining surface was occupied, not by grass, but by small weeds of the rosette type. It is questionable, indeed, whether we should include this field in a paper that has otherwise to do exclusively with grassland; but, since it was not under cultivation and was grazed, we admit it, and regard it with some interest as the exception that proves the rule.

To return now to our main theme, this rapid consideration of other fields shows that the composition of the larval population of Sheep's Green, as illustrated in Fig. 4, is not peculiar to that field, but is representative of similar pastures in other parts of England. Only one field of those we have sampled has been found to diverge from it, and that exception carries little weight, being so different in its covering of vegetation that it can scarcely be considered a grassland pasture. We can therefore conclude that under old grass a population of wireworm larvae normally consists, throughout the year, of large numbers of small larvae, progressively smaller numbers of larger larvae, and relatively very few of those large wireworms that have hitherto usually been allowed to represent the population.

In summing up thus simply we have not overlooked the differences in the shape of our several histograms. It is apparent, for instance, that in Fig. 4 and in some of the histograms on the lower row of Fig. 7, the number of larvae between 2 and 6 mm. in length bears a different relation to the remainder of the population than the number of those larvae in Fig. 6 and in the histograms forming the upper row of Fig. 7. These differences can be explained, but the explanation lies outside the limits of this paper, in which we are concerned with a census of wireworm populations in respect of their size and their general composition, not with any one of the many factors which operate to shape and control those populations.

To this point in our analysis of wireworm popu-

lations we have restricted our attention to the larvae. It would be natural now to extend our account to the eggs, the pupae, and the adults, and to proceed to a description of the population as a whole. As soon as we attempt to do so, however, we are confronted by serious complications which are due to the intrusion of the time factor. Each of those stages has only a seasonal occurrence. Adults, of course, may be found at almost all times of the year, including all four stages of the insect, the eggs are being laid, the pupae are being formed, the adults are dying, and the whole population is in such a state of rapid development and change that it would be of little use to consider it, as we have considered the larval population, from a static point of view. To analyse a complete wireworm population, in the sense of a population including all stages, it is essential to investigate, not so much its composition

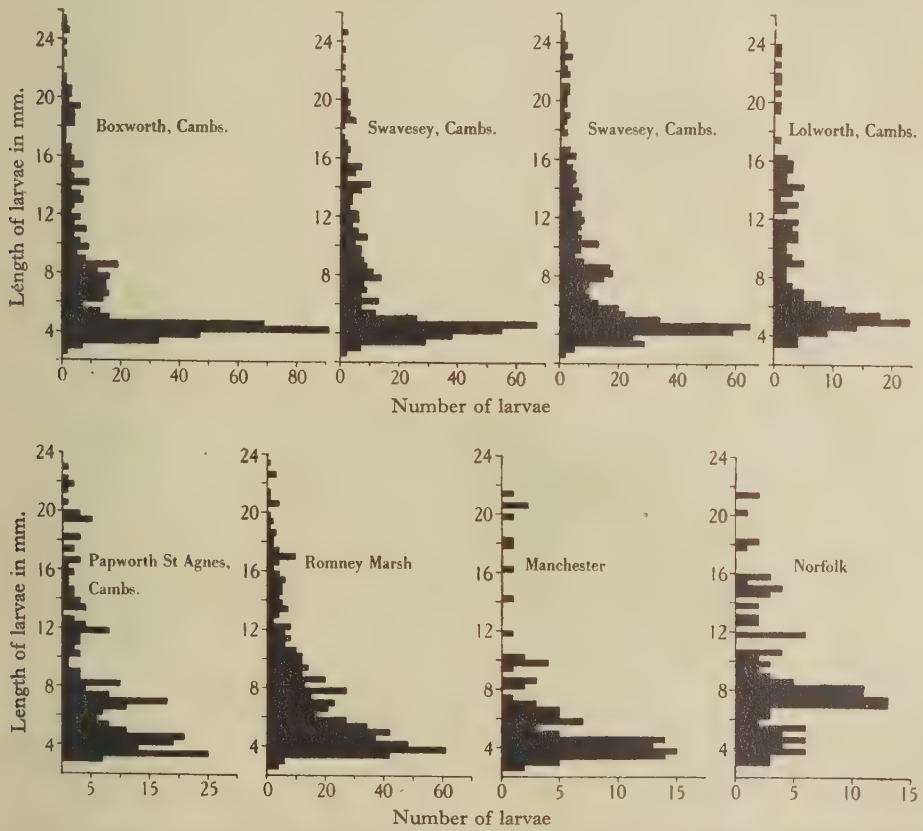


Fig. 7. The composition of the *Agriotes* population of five Cambridgeshire fields and of fields in three other parts of England.

but they are scarce from the middle of June to the middle of August, and absent from our collections during the month of July. Pupae occur during a period of about 10 weeks, from late in June until the end of August. Eggs have a still shorter season of about 7 weeks, from the end of June until the middle of August. During 9 months of the year, therefore, the wireworm population consists exclusively of larvae and adults, and has a relatively stable structure. But during the few weeks of the summer when it might conceivably be possible to find a population at any particular moment, as its development over a period of time.

We do not wish to become involved with the time factor and seasonal variations of the wireworm population in this paper, and therefore restrict ourselves to a few remarks about the stages other than the larval, in order to show how they fit into the general picture we have drawn of the larval population, and as an indication of the problems that must be solved before a thorough understanding of the whole population can be reached.

Taking our analysis of the larval population as a point of reference, it is clear that the number of eggs laid in any season must be large enough to provide the number of small larvae found in the succeeding months, allowing for the possible infertility or destruction of some of the eggs, and the continuous toll of death to which the larvae are subject from the moment of hatching. At the other end of the picture, the number of adult beetles found in the autumn of any year must be expected to be fewer than the number of large larvae approaching pupation in the preceding spring.

In general terms, both of these requirements are satisfied in our collections. In the months in which we have found eggs, they have occurred in numbers apparently sufficient to replenish the population of small larvae. Of adults, we have found a total of 249 in Sheep's Green and Spinney Pasture; which is 2% of the 12,107 larvae found there, a small enough return to accommodate a considerable larval mortality.

But as soon as we leave general terms and attempt to deal with the whole population more precisely, we find need of much more information than has yet been published. What proportion of the small larvae in a population come from eggs laid the previous season? How many of the large larvae in a population will pupate and transform into adults in a particular year? Until those questions, and others, can be answered, a balance sheet of the complete wireworm population cannot be drawn up.

Those questions cannot be answered with any degree of accuracy until the composition of the larval population has been studied season after season throughout a period of years, and with reference to the complications of growth and ecdysis and metamorphosis and death. Such a study has not hitherto been made, but some of the materials for it have begun to accumulate.

Discussion

Numbers are so obviously important in the wireworm problem, and so many people have counted wireworms for so long, that it is a matter for curiosity that the first quantitative description of a wireworm population should bear the date of this paper. That the work has not been done before is no doubt due to the difficulty of obtaining complete populations of insects from such an environment as the soil. Perhaps the most important contribution of this paper to the science of Entomology as a whole is our description of a method that provides for the collection of complete populations of insects from soil samples.

Although our method has been developed in response to the needs of the wireworm problem, its use is by no means confined to the collection of wireworms. Without any modification it can be applied to the recovery of arthropods from a variety of media. We have used it ourselves for sorting the inhabitants of an ant hill, and have made it available to workers investigating the sheep tick and the carrot fly. It is being modified, under our supervision, for the extraction of insect and other material from aquatic environments, including the mud on the bottom. In recent months it has been developed in another direction, to reduce the time and labour required in the examination of each sample, and we expect that the new version will make large-scale surveys compatible with scientific accuracy. The method, and particularly that part of it which separates chitin from vegetation, seems to us to provide a powerful tool for investigating a number of problems in economic entomology; and we hope also to see it used in a wide variety of ecological studies.

The first effect of the application of our method to the wireworm problem has been greatly to increase the known size of the population. Among the earliest published estimates of wireworm numbers are those of Morris (1920, 1922) who, in the course of a general study of the soil fauna, found 123,286 *Agriotes* per acre in pasture land in Cheshire, and 198,653 Elateridae per acre in arable land at Rothamsted. At the same period, Roebuck (1924) published observations made during the preceding 10 years, and recorded infestations of permanent grassland ranging from 100,000 to 900,000 per acre, with an average of about 300,000. During the decade 1930–40, it appears from the useful review of Thomas (1940, pp. 6–7) that populations of wireworms of several genera were considered high when they numbered 500,000 per acre. After the outbreak of war, the increased attention paid to wireworm infestations led to a sudden rise in the estimates. The national wireworm survey found the mean population of the Eastern province (including the Cambridge district) to be 627,000 wireworms per acre (Finney, 1941), and in the south-eastern province, where the average was 1,025,000 per acre, Jary (1942) found fields which exceeded three millions per acre. Still more recently, the use of our method has given much higher figures. From 240 4 in. samples taken at Spinney Pasture, twenty in each month of 1942, we collected 3880 larvae, pupae and adults of *Agriotes*. Applying the usual factor, this indicates a population of 8,100,000 per acre. The highest field population we have met is that from Romney Marsh (9 in Table 1) which, on the basis of twenty 4 in. samples taken to a depth of a foot, is 10,100,000 *Agriotes* per acre, together with other Elateridae. Over small areas, the density of wireworm populations may far exceed this figure. From one square-foot sample taken in Sheep's Green on 1 May 1941, we obtained 567 *Agriotes*, which represents a population of 24,700,000 per acre.

These numbers lead one to think of the population

of insects of all kinds to be found in soil. The information that was available on this subject about 20 years ago was summarized by Waksman (1927, chap. XIV), who gives 3,586,000 insects per acre in pasture and 7,727,000 per acre in a manured arable plot as the highest figures then known; both of them due to the work of Morris (1920, 1922). Since that time, much higher populations have been found. Ladell (1936) has reported observations made by Baweja which indicate populations of 47·9 million insects per acre in fallow land, and 95·6 millions per acre under new grass; and in some of his experimental plots at the Rothamsted Experimental Station, Baweja (1939, Table 7) found a mean population of the order of 105 million insects per acre. The densest population of soil insects that has yet been recorded appears to be that of Ford (1935) who, on the basis of sixteen samples 3 in. square and 9 in. deep, estimates that there were 244,894,320 insects per acre in the soil of an Oxfordshire meadow; of which 239·8 millions were Collembola and only five millions were other kinds of insects.

If the effect of our new method of extraction on known populations of wireworms is anything to go by, these numbers, large as they are, still tell only part of the story. We make no claim to have collected all the Collembola in our samples, nevertheless we found 6560 Arthropleone Collembola in the twenty samples taken from Spinney Pasture in May 1943; a figure which indicates that there were at least 164 millions per acre in the field in a month when, according both to Ford (1935) and to Thompson (1924), the soil Collembola are at or near their lowest ebb. To stray to other arthropods, in the same set of samples we counted 8707 mites, so that the acarine population of Spinney Pasture was at that time at least 218 millions per acre. Bearing in mind that our present apparatus was not designed for things so small as nymphal Collembola and mites, it would appear that, when thorough methods are applied to the whole arthropod fauna of the soil, populations of a density as yet unheard of will be discovered. To the arthropods must be added an average for eight regions of 338 million nematodes per acre (Steiner & Heinly, 1922); up to 600 million limnicole Oligochaeta per acre (Jegen, 1920); and estimates of 6–15 thousand Protozoa per gram of garden soil (Waksman, 1916). As numbers, these estimates carry little weight, since on the one hand they befuddle the imagination and on the other they are unreliable for quantitative work; but as ideas, contributing to a point of view, they are important. They forbid the zoologist to view the soil as an inert medium of inorganic particles, of direct interest only to the geologist, botanist and gardener, and lead him to see it as actually composed in large part of seething animal life.

But these considerations of the enormous population of the soil must not be allowed to divert us from our view that for a scientific approach to the wireworm problem, the size of the population is less important than its composition. The principal matter that we have wished to put forward in this paper is the idea of the preponderance of small larvae in wireworm populations living under long-established grass. That considerable numbers of small larvae must be present where so many large ones are found may have been evident; but by obtaining, counting and measuring them, we have provided the first quantitative description of natural wireworm populations. That description is epitomized in our histograms, and particularly in Fig. 4.

The consequence of that description, like the effect of our method, is to open up a whole series of new questions to answer. Why are there so many small wireworms and so few large ones? If the answer is that the larger larvae have had longer to die, we must ask how much longer; that is, what is the relation in these populations between size and age? In any case, what destroys the larvae, and when, and are those destructive agents equally pressing on the small larvae and on the large? Can any of the destructive agents be assisted and so bring about control? Some of these questions we shall try to answer in later papers in this series.

One problem we should like to raise, which we shall not ourselves be able to investigate, is the question of what the small larvae feed on, and their effect upon a crop. If the small larvae are unable to attack crops, they can be considered merely as potential dangers which do not become actual pests until they have grown up. But if the small larvae do attack the roots of crops it is possible that they make up in numbers what they lack in size and feeding capacity. If, for instance, we assign a value of 1 to the injury caused by each wireworm under 6 mm. long; an injury factor of 4 to each larva between 6 and 10 mm.; of 7 to those between 10 and 14 mm.; and of 10 to the larvae over 14 mm. long; then, in Sheep's Green, the damage done by the four groups would be 3834, 6676, 4032 and 4280 units respectively; and the effect of larvae less than 10 mm. long would be greater than that of the larvae over 10 mm. It may be that even in agricultural practice we cannot afford to neglect the small larvae; in scientific work on the wireworm problem certainly we cannot.

In addition to the acknowledgements made in the text, we must here thank several persons who have helped our work. We are particularly indebted to Prof. J. Gray, F.R.S., for allowing us to carry on this work in his Department; and we should also like to thank several members of the staff of the Zoological Laboratory for their help. Dr F. Kidd, Director of the Low Temperature Research Station, has kindly provided us with facilities for freezing soil samples in his refrigerating rooms. The encouragement and advice of Mr J. C. F. Fryer, O.B.E., has

been of great value to us. Dr J. E. Harris worked with us during the summer of 1940, when we were developing our method, and contributed materially to its success. The increased scale of our later work has been made possible by a grant from the Agricultural Research Council, which has provided us with materials, transport, and assistance. Of those who have at different times been employed under that grant, we should like especially to thank J. F. Blacklocks, B.Sc., F. Raw, B.Sc., and I. H. Mills, B.Sc., for their loyal and efficient services.

REFERENCES

Baweja, K. D. (1939). Studies of the soil fauna, with special reference to the recolonization of sterilized soil. *J. Anim. Ecol.* **8**, 120–61.

Finney, D. J. (1941). Wireworm populations and their effect on crops. *Ann. appl. Biol.* **28**, 282–95.

Ford, J. (1935). The animal population of a meadow near Oxford. *J. Anim. Ecol.* **4**, 195–207.

Jary, S. G. (1942). Wireworms and crop rotation. *Ann. appl. Biol.* **29**, 150–5.

Jegen, C. (1920). Die Bedeutung der Enchytraeiden für die Humusbildung. *Landw. Jb. Schweiz*, **34**, 55–71.

Ladell, W. R. S. (1936). A new apparatus for separating insects and other arthropods from the soil. *Ann. appl. Biol.* **23**, 862–79.

Miles, H. W. & Cohen, M. (1938). Investigations on wireworms and their control. *Ann. J. Roy. Lancs. agric. Soc.* 1938, pp. 58–79.

Morris, H. M. (1920). Observations on the insect fauna of permanent pasture in Cheshire. *Ann. appl. Biol.* **7**, 141–55.

Morris, H. M. (1922). The insect and other invertebrate fauna of arable land at Rothamsted. *Ann. appl. Biol.* **9**, 282–305.

Roebuck, A. (1924). Destruction of wireworms. *J. Minist. Agric.* **30**, 1047–51.

Steiner, G. & Heinly, H. (1922). The possibility of control of *Heterodera radicola*. *J. Wash. Acad. Sci.* **12**, 367–86.

Thomas, C. A. (1940). The biology and control of wireworms. *Bull. Pa St. Coll.* no. 392, 90 pp.

Thompson, M. (1924). The soil population. An investigation of the biology of the soil in certain districts of Aberystwyth. *Ann. appl. Biol.* **11**, 349–94.

Waksman, S. A. (1916). Studies on soil Protozoa. *Soil Sci.* **1**, 135–52.

—— (1927). *Principles of soil microbiology*. xxviii +897 pp. Baltimore.

(*Received* 24 *June* 1943)

The use of toxic polynitro derivatives in pest control

II. The estimation of dinitro-*o*-cresol in winter washes

By R. F. Batt, H. Martin and R. L. Wain, *Research Station, Long Ashton, Bristol*

The estimation of dinitro-*o*-cresol in winter washes consisting of petroleum oil, the cresol and emulsifier, must first involve the separation of the cresol from the other constituents present. A method applicable to the cases of emulsions compounded with various types of emulsifier failed when sulphite lye was present, and this was shown to be due to the action on the cresol of reducing sugars in alkaline solution. Avoiding an alkaline extraction until these interfering substances had been effectively removed led to an alternative method by which it was possible to separate D.N.C. in this type of emulsion.

The results obtained demonstrate that the methods described are satisfactory for the routine estimation of D.N.C. in petroleum oil/D.N.C. emulsions.

The use of 3:5-dinitro-*o*-cresol (D.N.C.) as a constituent of petroleum oil washes was reviewed in the first paper of this series (Wain, 1942), in which two methods for estimating the compound itself were described. The present paper is concerned with the estimation of D.N.C. in the stock emulsion to which the ovicide is compounded for the grower's use. The method sought has been one which will permit the identification as well as the estimation of the cresol and which will allow a simultaneous estimation of the oil and its separation for the examination of the various physical criteria by which the oil is characterized. The latter object is achieved, in the case of stock emulsions of petroleum oils, by the solvent extraction of the oil from the diluted emulsion broken by alkali (Martin, 1935). When this procedure is applied to the D.N.C.-petroleum stock emulsion the cresol is retained as cresylate in the alkaline aqueous

layer, from which it can be separated by solvent extraction after acidification. As the cresol obtained by this method will be contaminated by other acidic constituents of the stock emulsion an alternative method was first examined, taking advantage of the volatility of D.N.C. in steam.

EXPERIMENTAL

Method A. *Alkaline extraction method*

To reduce sampling errors in the exploratory work, a known weight of pure D.N.C. was added direct to the separating funnel containing known amounts of oil, water and emulsifier. The oil used conformed to the Grade E specification (Martin, 1935).

A weighed amount (about 100 g.) of the stock emulsion was transferred to a 1500–2000 ml. separating funnel, the cresol, 400 ml. water, 25 ml. 10% sodium hydroxide and 400 ml. ether (*d.* 0·730) added and the mixture well shaken. After standing overnight the aqueous layer was removed and the ether solution washed by shaking with 50 ml. lots of water until the aqueous extract was colourless. These extracts were combined with the first aqueous fraction. After withdrawal of the ether layer containing petroleum oil, the combined alkali washings were placed back in the funnel, re-extracted with 200 ml. ether, the extract being washed with 50 ml. lots of water until the aqueous layer was colourless. The ether layer was combined with the main ether/oil solution and reserved for the estimation of oil content. The aqueous washings were then added to the main bulk and transferred to a 1 l. round-bottomed steam-distillation flask; about 10 g. sodium sulphate (to raise the boiling point of the solution) and a few pieces of porous pot were added followed by 20 ml. 50% sulphuric acid. An efficient splash head and a Liebig condenser with at least 2½ ft. cooling tube were connected, and the contents of the flask distilled, gently at first until dissolved ether was removed, then vigorously until some 200 ml. only remained. During this process the bulk of the D.N.C. distilled over with the steam. A steam generator was then fitted and the residual D.N.C. removed by passing a brisk current of steam, until the condensate in the splash head was colourless. By this means, a complete removal of D.N.C. was obtained with a distillate not exceeding 1 l., whereas if the steam generator is connected at the outset and no sodium sulphate is used the volume of distillate required is much greater.*

The condenser was now disconnected and any D.N.C. in the tube washed into the receiver with ether. The contents of the receiver were transferred to a separating funnel and extracted with three successive 250 ml. lots of ether. After distilling off the ether the cresol was transferred to a 200 ml. volumetric flask with alcohol and made up to the mark.

25 ml. portions of this solution were then pipetted out for triplicate and duplicate analyses by the volumetric (back titration) and gravimetric methods described in Part I of this series.

The results, expressed as the percentage recovery of D.N.C., are assembled in Table 1, in which the various emulsifiers present are briefly described.

TABLE 1. *Estimation of* D.N.C. *in mixtures of oil, water and emulsifying agent*

Emulsifier	% D.N.C. recovered: By titration (av. of triplicates)	% D.N.C. recovered: By nitron method	M.p. of nitron complex ° C. uncorr.
Methyl cellulose	99·65	97·38 97·34	217–219
	97·50	96·16 95·92	217–219
Size	99·08	97·52 97·63	217–219
	95·54	94·34 94·58	217·5–219
P_1	99·95	96·54 96·63	217–218·5
	99·33	97·29 97·45	217–218
P_2	98·72	97·86 97·29	217–218·5
	98·47	97·16 97·27	218–219
P_3	98·56	97·83 98·02	218–219
	97·59	93·40 93·56	219
	98·93	93·50	217·5–219
	99·56	—	—
Sulphite lye	71·85	61·76 62·04	215–218
	90·12	79·27	217–218·5
	85·67	75·68	217·5–219
	86·96	82·79	218–219·5

P_1, P_2 and P_3 are proprietary emulsifiers, P_1 consisting of the sodium salts of secondary alcohol sulphates of the type $RR'.CH.O.SO_3Na$, P_2 and P_3 being emulsifiers based on degraded proteins.

The recovery of the cresol is satisfactory with all the emulsifiers tested, with the important exception of sulphite lye, which has been found well suited for the preparation of D.N.C.-petroleum emulsions (Kearns & Martin, 1941).

Reasons for the failure of method A in presence of sulphite lye

The reasons for the unsatisfactory recovery of the cresol from the alkaline layer in presence of sulphite lye are presumably to be found in sulphite lye itself. Sulphite lye, which is a by-product of manufacture of cellulose from wood pulp by the sulphite process,

* In a direct steam distillation on the acidified emulsion, D.N.C. still remained dissolved in the oil after 7 l. of distillate had been collected.

is complex, containing lignosulphonates, sulphites, free sulphur dioxide, carbohydrates and their acidic derivatives and volatile organic acids (Howard, 1934). It is clear that since the iodide/iodate method for estimation of D.N.C. (Wain, 1942) depends on the acidic character of the cresol, contamination of the extract with acidic substances from the lye would affect the result positively. The gravimetric method, however, is not likely to be influenced by these substances. It will be noted in Table 1 that the results with the gravimetric are lower than with the iodometric method to an extent greater with sulphite lye than with any other emulsifier, which suggests contamination with steam-volatile acids. The steam distillate from acidified sulphite lye, although giving no precipitate with the nitron acetate reagent, contained a considerable amount of sulphurous acid. Formic, acetic and lactic acids were shown to be present by qualitative tests. The presence of these acids, while accounting for the difference between the gravimetric and iodometric results, is no explanation for the low gravimetric figures.

A direct test was made of the effect of sulphur dioxide on D.N.C., first, in ether solution. A rapid stream of sulphur dioxide was passed through a solution of the cresol in ether. After 30 min. the ether was distilled off. As the melting point of the residue (84–85°) showed no depression when mixed with an authentic specimen of D.N.C. no decomposition products of the latter were present. Secondly, a suspension of 1·5 g. D.N.C. in 600 ml. water was treated with a rapid stream of sulphur dioxide for 90 min. The percentage recovery of the cresol by ether extraction with three aqueous washings of the extract was 103·6 and 97·07 % when estimated by the iodometric and gravimetric methods respectively. The high result in the former method can be attributed to sulphurous acid contamination, while the good recovery by the nitron method and the melting point of the complex (218–219° C. uncorr.) shows that the extent of decomposition of the cresol by this treatment is negligible.

A second possible reason for the low recovery from sulphite lye is the decomposition of the cresol by the reducing sugars present in the latter. Accordingly, 1 g. D.N.C. was dissolved in an excess of 2 % sodium hydroxide, 5 g. glucose added and the solution boiled for 30 min. On acidifying the cooled deep red solution, no immediate precipitate was produced and a steam distillate from this solution contained only a small amount of D.N.C. In a blank experiment, using no glucose, the cresol was precipitated immediately on acidification. These results indicate that D.N.C. undergoes reaction with boiling alkaline glucose solution, a process which is presumably one of reduction.

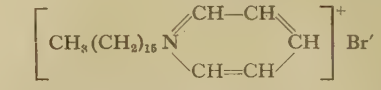
In experiments similar to the above, using D.N.C. with aqueous glucose and D.N.C. with glucose and *N* sulphuric acid, no reduction took place, indicating that alkaline glucose is the effective reducing agent. That the effect of alkali was more than to bring D.N.C. into solution, thus facilitating reduction by glucose, was disproved, since neither a solution of the cresol in aqueous alcohol nor an aqueous solution of the sodium salt (in absence of free alkali) was affected by boiling with glucose. The reduction of D.N.C. by alkaline glucose, a time reaction, was shown to occur at laboratory temperatures.

Sulphite lye gave substantially similar results to glucose when free alkali was present. Thus when 2·5 g. D.N.C. in 300 ml. 1 % sodium hydroxide was treated with 20 ml. concentrated lye and allowed to stand for 1 week in the laboratory, no trace of D.N.C. was obtained on steam distilling the acidified solution. The alkaline method of extraction of D.N.C. from the stock emulsion (method A), while successful with other emulsifying agents, failed when sulphite lye was present, because the D.N.C. was slowly destroyed in the alkali washings even at ordinary temperatures.

It is therefore necessary, in order to extract D.N.C. from stock emulsions containing sulphite lye or reducing sugars, either to avoid an alkaline extraction entirely or to ensure the absence of reducing sugars when the alkaline extraction is made. An ether extraction of D.N.C. and oil under acid conditions should separate the cresol from sugars which are difficultly soluble in ether. This was the basis of method B, applicable to the case of stock emulsions responding to tests for reducing substances. The presence of the latter may be detected by the formation of red cuprous oxide when a little of the emulsion is warmed with twice its volume of Fehling's solution. Sulphite lye is suspected if, when about 1 ml. of the emulsion is boiled in a test-tube with 1 ml. 5 % sulphuric acid, a blue colour is produced on starch-iodate paper held at the mouth of the tube.

Method B. *Acid extraction method*

Shaking the acidified emulsion with ether and sodium chloride, although effecting a separation, was unsatisfactory, there being much scum at the interface, which made it difficult to separate the two phases completely. In attempts to obtain a rapid clean break of the acidified emulsion the effects of other materials were studied. Phenol was promising in this respect, but by far the most effective were compounds of the quaternary ammonium salt type where one radical (*R*) was a long hydrocarbon chain, i.e. $[RNR_1R_2]^+X'$. The compound selected for use in this work was cetyl pyridinium bromide



which is present in the proprietary product 'Fixanol'. Various other materials of this type, however, were equally effective.

A weighed sample (100 g.) was placed in a separating funnel (1500–2000 ml.), 400 ml. water added followed by 400 ml. ether and 50 ml. 5% sulphuric acid. After shaking, a filtered solution of 5% 'Fixanol' was added, 2 ml. at a time, shaking after each addition until the emulsion broke cleanly. About 10 ml. were usually required. The aqueous layer was then withdrawn and the ether solution filtered from the funnel into a dry flask. The aqueous phase was re-extracted twice with 200 ml. quantities of ether, the extracts being filtered as before and combined with the main bulk. The filter paper was then washed with ether, the washings also being combined with the main ether solution. The aqueous phase was discarded.

TABLE 2. *Estimation of* D.N.C. *in* D.N.C./*petroleum oil emulsions compounded with sulphite lye*

% D.N.C. recovered		
By titration (av. of triplicates)	By nitron method	M.p. of nitron complex ° C. uncorr.
99·26	96·29 96·36	218–219·5
98·68	96·54 96·68	217·5–219
98·22*	95·79*	218·5–219·5

* Chloroform was used for extraction throughout.

The ether solution containing oil and D.N.C. was placed back into the cleaned separating funnel, 400 ml. water and 30 ml. 10% sodium hydroxide added and the mixture well shaken. From this stage onwards to acidification it was necessary to separate the sodium salt of the cresol from the ether/oil phase as quickly as possible. The ether solution was washed with 50 ml. quantities of water until the extract was colourless, these washings being combined with the main bulk. The subsequent procedure, including steam distillation, was exactly as described previously. The ether extract of the steam distillate, which may contain traces of volatile acids, was shaken with 10 ml. water. If colourless, the aqueous layer was withdrawn and the ether washed with further 10 ml. lots of water until the aqueous extract was slightly yellow. The aqueous washings were extracted once with 50 ml. ether, the extract being combined with the main bulk. Ether was then removed by distillation, the cresol dissolved in alcohol and estimated in this solution in the usual manner.

In testing the recovery of D.N.C. by this method, weighed amounts of the cresol were added to the requisite quantities of oil emulsion made up with sulphite lye. The results are given in Table 2.

DISCUSSION

The results (Tables 1 and 2) establish that D.N.C. in stock emulsions can be estimated with sufficient accuracy for routine work by the methods described.

The alkaline extraction method, while satisfactory in the presence of other emulsifiers, fails with emulsions compounded with sulphite lye, and it was established that this was due to the action in alkaline solution of reducing sugars present in the lye. In the acid extraction method these interfering substances were effectively removed from the cresol and it became possible to extract with safety as cresylate.

In addition to yielding reducing sugars, sulphite lye can also give rise to acid contamination of the extracted cresol. That the nitron acetate reagent yielded no precipitate with the steam distillate from acidified sulphite lye, however, indicates that the gravimetric estimation is unaffected by this emulsifier.

Although sulphur dioxide was shown to be without reducing action on D.N.C. the presence of sulphurous and other acids at the titration stage would affect the result positively. The procedures adopted, however, viz. ether extraction, steam distillation, ether extraction with aqueous washing of the extract and removal of ether, all combine to free the cresol from acid contamination. Even when D.N.C. was extracted from a solution saturated with sulphur dioxide, the increase by the volumetric method due to sulphurous acid contamination was not more than 5%, and here the steam distillation stage was omitted. It may therefore be concluded that the relatively small amount of sulphur dioxide liberated during the analysis of a stock emulsion compounded with lye is effectively removed. This is in agreement with the results in Table 2, which show that no appreciable interference by sulphurous or other acids occurs at the titration stage.

This work was facilitated by a grant from the Agricultural Research Council.

REFERENCES

BUSCH, M. (1905). Ueber Endimino-triazole. *Ber. dtsch. chem. Ges.* **38**, 856.

HOWARD, G. C. (1934). Utilization of sulphite liquor. *Industr. Engng Chem.* **28**, 614.

KEARNS, H. G. H. & MARTIN, H. (1941). The preparation of dinitro-ortho-cresol emulsions. *Rep. agric. hort. Res. Sta. Bristol for* 1940, p. 55.

MARTIN, H. (1935). The standardization of petroleum and tar oils and preparations as insecticides. *Ann. appl. Biol.* **22**, 334.

WAIN, R. L. (1942). The use of toxic polynitro compounds in pest control. I. The estimation of dinitro-*o*-cresol and dinitro-*o*-*cyclo*hexylphenol. *Ann. appl. Biol.* **29**, 301.

APPENDIX

Recovery of nitron. This substance, which is expensive, can be recovered and used again. The filtrate in the gravimetric method containing nitron acetate is treated with dilute nitric acid and the nitron nitrate which separates is filtered, washed with water, dried and stored in a residue bottle in which nitron-D.N.C. complex is also collected. When sufficient residues have accumulated, transfer to a separating funnel, shake with 2% sodium hydroxide and chloroform, adding water until both phases are clear after shaking. Withdraw the lower chloroform layer and re-extract the aqueous layer twice with chloroform. Discard the aqueous solution, wash the combined chloroform extract twice with water, withdraw into a dry flask and allow to stand over freshly ignited sodium sulphate. (The use of sodium hydroxide as drying agent as recommended by Busch (1905) is unsatisfactory, some decomposition occurring with the formation of an isocyanate.) After filtering the solution, concentrate the filtrate to a small volume. On addition of a few drops of 40–60 petroleum and a seed of nitron, the base crystallizes in small bright yellow needles which are filtered off and washed on the filter with a little chloroform.

In a typical recovery using 1·54 g. nitron nitrate and 3·70 g. nitron-D.N.C. complex, 2·60 g. pure nitron m.p. 189° (*d.*) were obtained.

(*Received* 25 *July* 1943)

The application of the probit method to toxicity test data adjusted for mortality in the controls

By D. J. Finney, M.A., *Rothamsted Experimental Station, Harpenden, Herts*

When data from toxicity tests have to be adjusted for a mortality rate amongst untreated controls, three modifications to the usual method of probit analysis need to be considered. The full solution is more laborious than that ordinarily used, as it requires the estimation of this mortality rate from the whole of the data. When the rate is not high (e.g. up to 20%) a satisfactory approximation may be obtained by a simple alteration in the weighting coefficients, especially if the precaution is taken of using two or three times as many test organisms as controls as for any dosage of the poison, thus decreasing the relative value of the information on natural mortality contributed by the treated batches. The modified weighting coefficients are tabulated for values of the natural mortality from zero to 20%. An example of the more complex calculations required for the full maximum likelihood solution is discussed in detail.

Introduction

In assessing the proportion of a batch of insects killed as a result of the application of poison, allowance must frequently be made for the fact that a natural mortality rate is operating in addition to the effect of the poison. Similarly, in ovicidal tests, if it is not possible to distinguish between fertilized and unfertilized eggs at the start of the test, the percentage failing to hatch must be adjusted for the percentage unfertilized in the population from which the test eggs were taken. In germination tests of fungicides the situation is again similar.

The formula for this adjustment is generally attributed to Abbott (1925), though it had been used earlier for the same purpose by Tattersfield & Morris (1924), and is in fact an application of a well-known rule for the combination of independent probabilities. If a proportion, C, of the test organisms would be found dead at the end of the experiment without any application of poison, and if a strength of poison is used sufficient to kill a proportion P of those which would otherwise survive, the total death-rate expected is

$$P' = P(1 - C) + C.$$

If the observed proportion dead is p', a corresponding estimate of p, the proportion whose death is caused by the poison, is given by 'Abbott's formula'

$$p = (p' - C)/(1 - C).$$

This argument is only valid when the poison and the natural mortality operate independently; any

interaction between the two causes of death destroys its validity.

The usual practice in toxicity tests is to employ a series of different concentrations of the poison on separate batches of the test organism, a further batch being kept as controls, either untreated or sprayed with the spray fluid without any content of poison. The death rate amongst these controls is an estimate, c, of C, which estimate is used in Abbott's formula to give values for p at each concentration. The relationship between p and the concentration is then investigated by means of the standard method of probit analysis (Bliss, 1935; Fisher & Yates, 1943).

If there is no natural mortality to be considered, C and c are both zero and this procedure leads to the maximum likelihood solution for the normal sigmoid curve relating percentage kill to log concentration. For small values of C the result obtained will be very close to the maximum likelihood solution, provided that a reasonably large control batch is used so as to give a value of c free from gross sampling errors. The larger the value of C the more important are the effects of modifications in the maximum likelihood solution arising from three separate sources:

(1) The effective number of test organisms exposed to the poison is not n, the total number in a batch, but, on an average, $n(1-C)$.

(2) The weighting coefficient used in the standard probit analysis is $w=Z^2/PQ$, where $Q=1-P$ and Z is the ordinate of the normal curve cutting off an area P. In this expression PQ arises as the variance of a binomial frequency distribution, and when C is not zero the relevant distribution is that defined by the *total proportions* of dead and surviving, P' and Q'.

(3) In general, an estimate, c, of C must be used, and this estimate is subject to sampling errors. Most of the information from which this estimate is derived will come from the control batches, but results from low concentrations of poison producing total kills not much greater than C will also contribute to the available information and their contribution should be utilized in the maximum likelihood solution.

Of these three points, only (1) appears to have been appreciated in any published accounts of probit analysis. Thus Bliss (1939, p. 602) states 'Both the number of eggs exposed and the percentage kill have been corrected for mortality in the untreated controls', and examination of his correction shows the number of eggs tested at each concentration to have been reduced by the percentage mortality in the controls. This adjustment, altering the weights attached to each observation in the same proportion, does not affect the estimates of toxicity, but shows the precision of such estimates to be less than appears if the adjustment is ignored.

The effect of (2) is to reduce still further the weight to be attached to the probit of each observation. This reduction is not symmetrical about a probit value of 5, but gives much lower weights at low probits than at the corresponding high values. The effect of (3) is more complicated, and will be discussed later in this paper.

Approximate methods of analysis

The first two modifications may be combined by taking as the weighting coefficient

$$w=\frac{Z^2}{Q\left(P+\frac{C}{1-C}\right)}=\frac{Z^2}{PQ}\left(\frac{P}{P+C/(1-C)}\right),$$

and using c, the observed mortality among the controls, as an estimate of C. In many cases this adjustment to the weighting coefficient will suffice to give a satisfactory approximation to the maximum likelihood solution, the analysis then proceeding along the usual lines. The solution would be exact if the true value of C were known, and will be a close approximation if a relatively large batch of test organisms is used as control. Even with C as small as 0·05 the reduction in weight may be considerable and may be sufficient to remove an apparent heterogeneity of departures from the probit regression line found when no adjustment was made to the weights.

The ordinary weighting coefficients as tabulated, for example, by Fisher & Yates (1943, Table XI), correspond to the case $C=0$, and for any other value of C these must be multiplied by

$$\frac{P}{P+C/(1-C)}.$$

Table 1 gives w for values of C from 0·01 to 0·20; further values may be calculated as required.

Example. To find the value of w for an expected probit, Y, of 6·2, the mortality amongst the controls being 59%.

With $c=0{\cdot}59$,

$$c/(1-c)=1{\cdot}4390.$$

Also, from Table IX of Fisher & Yates, the mortality corresponding to a probit of 6·2 is 88·49%. Hence

$$\frac{P}{P+c/(1-c)}=0{\cdot}8849/(0{\cdot}8849+1{\cdot}4390)=0{\cdot}3808.$$

The weighting factor given in Table XI for a probit of 6·2 is 0·37031 and therefore

$$w=0{\cdot}37031\times 0{\cdot}3808=0{\cdot}1410.$$

When c is zero the same weighting coefficient is taken for a probit of 3·8 as for 6·2, but with $c=0{\cdot}59$ a probit of 3·8 has $P=0{\cdot}1151$ and

$$\frac{P}{P+c/(1-c)}=0{\cdot}0741.$$

Hence $$w=0{\cdot}37031\times 0{\cdot}0741=0{\cdot}0274.$$

This approximate analysis, apart from a little additional labour in calculating the weights, is no

TABLE 1. *Table of Q/Z and weighting coefficients*

		Natural mortality, C										
Y	*Q/Z*	·00	·01	·02	·03	·04	·05	·06	·07	·08	·09	·10
1·1	5034·0	·00082	·00000	·00000	·00000	·00000	·00000	·00000	·00000	·00000	·00000	·00000
1·2	3425·0	·00118	·00001	·00000	·00000	·00000	·00000	·00000	·00000	·00000	·00000	·00000
1·3	2354·0	·00167	·00002	·00001	·00001	·00000	·00000	·00000	·00000	·00000	·00000	·00000
1·4	1634·0	·00235	·00004	·00002	·00001	·00001	·00001	·00001	·00000	·00000	·00000	·00000
1·5	1146·0	·00327	·00007	·00004	·00002	·00002	·00001	·00001	·00001	·00001	·00001	·00001
1·6	811·2	·00451	·00015	·00007	·00005	·00004	·00003	·00002	·00002	·00002	·00002	·00001
1·7	580·2	·00614	·00028	·00014	·00009	·00007	·00006	·00005	·00004	·00003	·00003	·00003
1·8	419·1	·00828	·00053	·00027	·00018	·00013	·00011	·00009	·00007	·00006	·00006	·00005
1·9	305·8	·01105	·00097	·00050	·00034	·00025	·00020	·00017	·00014	·00012	·00011	·00010
2·0	225·3	·01457	·00172	·00090	·00061	·00046	·00036	·00030	·00026	·00022	·00020	·00017
2·1	167·69	·01903	·00297	·00159	·00108	·00082	·00065	·00054	·00046	·00040	·00035	·00031
2·2	126·02	·02458	·00496	·00274	·00188	·00142	·00114	·00095	·00081	·00070	·00062	·00055
2·3	95·63	·03143	·00803	·00456	·00317	·00241	·00194	·00162	·00138	·00121	·00106	·00095
2·4	73·28	·03977	·01286	·00739	·00521	·00400	·00324	·00271	·00232	·00202	·00179	·00160
2·5	56·70	·04979	·01895	·01161	·00832	·00646	·00525	·00441	·00379	·00332	·00294	·00264
2·6	44·288	·06168	·02763	·01768	·01292	·01014	·00831	·00702	·00606	·00531	·00472	·00424
2·7	34·923	·07563	·03895	·02605	·01947	·01548	·01280	·01088	·00943	·00830	·00740	·00666
2·8	27·797	·09179	·05316	·03719	·02847	·02297	·01918	·01642	·01431	·01265	·01131	·01021
2·9	22·330	·11026	·07044	·05147	·04037	·03309	·02794	·02411	·02115	·01879	·01687	·01527
3·0	18·101	·13112	·09080	·06912	·05557	·04631	·03957	·03445	·03043	·02719	·02452	·02228
3·1	14·802	·15436	·11419	·09023	·07432	·06298	·05449	·04790	·04263	·03832	·03473	·03170
3·2	12·211	·17994	·14046	·11476	·09670	·08332	·07300	·06481	·05814	·05261	·04795	·04397
3·3	10·159	·20773	·16935	·14249	·12263	·10736	·09525	·08541	·07726	·07039	·06453	·05947
3·4	8·521	·23753	·20056	·17308	·15184	·13494	·12116	·10973	·10008	·09182	·08469	·07846
3·5	7·205	·26907	·23373	·20611	·18392	·16571	·15050	·13760	·12652	·11690	·10848	·10103
3·6	6·1394	·30199	·26842	·24107	·21836	·19921	·18283	·16867	·15631	·14541	·13575	·12711
3·7	5·2705	·33589	·30415	·27741	·25456	·23482	·21759	·20242	·18896	·17694	·16614	·15639
3·8	4·5571	·37031	·34043	·31453	·29186	·27187	·25409	·23819	·22387	·21092	·19915	·18840
3·9	3·9676	·40474	·37669	·35181	·32960	·30964	·29161	·27524	·26031	·24665	·23409	·22250
4·0	3·4770	·43863	·41237	·38864	·36707	·34739	·32937	·31279	·29749	·28334	·27020	·25797
4·1	3·0665	·47144	·44691	·42438	·40362	·38441	·36661	·35005	·33460	·32017	·30666	·29397
4·2	2·7206	·50260	·47973	·45844	·43858	·42000	·40259	·38623	·37085	·35634	·34264	·32969
4·3	2·4276	·53159	·51029	·49024	·47134	·45350	·43662	·42063	·40546	·39105	·37735	·36430
4·4	2·1780	·55788	·53806	·51924	·50134	·48430	·46805	·45255	·43774	·42357	·41002	·39702
4·5	1·9640	·58099	·56257	·54495	·52806	·51187	·49633	·48140	·46705	·45325	·43996	·42716
4·6	1·7797	·60052	·58341	·56694	·55106	·53574	·52095	·50666	·49286	·47951	·46659	·45409
4·7	1·6202	·61609	·60022	·58485	·56996	·55551	·54150	·52790	·51470	·50187	·48941	·47729
4·8	1·4814	·62742	·61271	·59840	·58446	·57089	·55766	·54478	·53221	·51996	·50801	·49635
4·9	1·3599	·63431	·62069	·60737	·59436	·58164	·56921	·55704	·54514	·53350	·52210	·51094
5·0	1·2533	·63662	·62401	·61165	·59953	·58765	·57599	·56455	·55332	·54230	·53149	·52087
5·1	1·1593	·63431	·62266	·61120	·59994	·58886	·57796	·56724	·55669	·54631	·53609	·52604
5·2	1·0759	·62742	·61667	·60607	·59562	·58532	·57516	·56515	·55527	·54553	·53592	·52644
5·3	1·0018	·61609	·60618	·59639	·58672	·57717	·56773	·55841	·54919	·54008	·53108	·52219
5·4	0·9357	·60052	·59140	·58238	·57346	·56462	·55588	·54722	·53866	·53018	·52178	·51347
5·5	0·8764	·58099	·57263	·56434	·55612	·54797	·53990	·53189	·52396	·51609	·50829	·50056
5·6	0·8230	·55788	·55022	·54262	·53507	·52759	·52015	·51278	·50545	·49818	·49097	·48380
5·7	0·7749	·53159	·52460	·51765	·51075	·50389	·49708	·49030	·48357	·47688	·47024	·46363
5·8	0·7313	·50260	·49624	·48992	·48363	·47737	·47114	·46495	·45879	·45266	·44657	·44050
5·9	0·6917	·47144	·46567	·45993	·45422	·44853	·44287	·43723	·43162	·42603	·42047	·41493
6·0	0·6557	·43863	·43343	·42824	·42308	·41793	·41281	·40770	·40261	·39754	·39249	·38746
6·1	0·6227	·40474	·40006	·39540	·39075	·38612	·38150	·37690	·37231	·36774	·36318	·35863
6·2	0·5926	·37031	·36613	·36196	·35781	·35366	·34952	·34540	·34128	·33718	·33308	·32900
6·3	0·5649	·33589	·33218	·32847	·32477	·32108	·31740	·31372	·31006	·30640	·30274	·29910
6·4	0·5394	·30199	·29871	·29543	·29216	·28890	·28564	·28238	·27913	·27589	·27266	·26942
6·5	0·5158	·26907	·26619	·26331	·26044	·25757	·25470	·25184	·24899	·24613	·24329	·24044
6·6	0·4940	·23753	·23502	·23251	·23001	·22751	·22501	·22251	·22001	·21752	·21503	·21255
6·7	0·4739	·20773	·20556	·20339	·20122	·19905	·19689	·19473	·19256	·19041	·18825	·18609
6·8	0·4551	·17994	·17808	·17621	·17435	·17249	·17063	·16877	·16691	·16506	·16320	·16135
6·9	0·4376	·15436	·15277	·15118	·14960	·14801	·14643	·14484	·14326	·14168	·14010	·13852
7·0	0·4214	·13112	·12977	·12843	·12709	·12575	·12442	·12308	·12174	·12040	·11907	·11773
7·1	0·4062	·11026	·10914	·10802	·10689	·10577	·10465	·10353	·10241	·10129	·10017	·09905
7·2	0·3919	·09179	·09086	·08993	·08900	·08807	·08714	·08621	·08528	·08435	·08342	·08249
7·3	0·3786	·07564	·07487	·07411	·07334	·07258	·07181	·07105	·07029	·06952	·06876	·06800
7·4	0·3660	·06168	·06106	·06044	·05982	·05920	·05858	·05795	·05733	·05671	·05609	·05547
7·5	0·3543	·04979	·04929	·04879	·04828	·04778	·04728	·04678	·04628	·04578	·04528	·04478
7·6	0·3432	·03977	·03937	·03897	·03857	·03817	·03777	·03737	·03697	·03657	·03617	·03577
7·7	0·3327	·03143	·03112	·03080	·03048	·03017	·02985	·02954	·02922	·02891	·02859	·02828
7·8	0·3228	·02458	·02434	·02409	·02385	·02360	·02335	·02311	·02286	·02261	·02237	·02212
7·9	0·3134	·01903	·01883	·01864	·01845	·01826	·01807	·01788	·01769	·01750	·01731	·01712
8·0	0·3046	·01457	·01442	·01428	·01413	·01399	·01384	·01369	·01355	·01340	·01326	·01311
8·1	0·2962	·01104	·01093	·01082	·01071	·01060	·01049	·01038	·01027	·01016	·01005	·00993
8·2	0·2882	·00828	·00819	·00811	·00803	·00795	·00786	·00778	·00770	·00762	·00753	·00745
8·3	0·2806	·00614	·00608	·00602	·00596	·00590	·00583	·00577	·00571	·00565	·00559	·00553
8·4	0·2734	·00451	·00446	·00442	·00437	·00433	·00428	·00424	·00419	·00415	·00410	·00406
8·5	0·2665	·00327	·00324	·00321	·00318	·00314	·00311	·00308	·00305	·00301	·00298	·00295
8·6	0·2600	·00235	·00233	·00231	·00228	·00226	·00224	·00221	·00219	·00217	·00214	·00212
8·7	0·2538	·00167	·00166	·00164	·00162	·00161	·00159	·00157	·00156	·00154	·00152	·00150
8·8	0·2478	·00118	·00117	·00116	·00114	·00113	·00112	·00111	·00110	·00108	·00107	·00106
8·9	0·2421	·00082	·00081	·00080	·00080	·00079	·00078	·00077	·00076	·00076	·00075	·00074
9·0	0·2369	·00056	·00056	·00055	·00055	·00054	·00054	·00053	·00053	·00052	·00051	·00051

TABLE 1 (*continued*)

		Natural mortality, C									
Y	Q/Z	·11	·12	·13	·14	·15	·16	·17	·18	·19	·20
1·1	5034·0	·00000	·00000	·00000	·00000	·00000	·00000	·00000	·00000	·00000	·00000
1·2	3425·0	·00000	·00000	·00000	·00000	·00000	·00000	·00000	·00000	·00000	·00000
1·3	2354·0	·00000	·00000	·00000	·00000	·00000	·00000	·00000	·00000	·00000	·00000
1·4	1634·0	·00000	·00000	·00000	·00000	·00000	·00000	·00000	·00000	·00000	·00000
1·5	1146·0	·00001	·00001	·00001	·00000	·00000	·00000	·00000	·00000	·00000	·00000
1·6	811·2	·00001	·00001	·00001	·00001	·00001	·00001	·00001	·00001	·00001	·00001
1·7	580·2	·00002	·00002	·00002	·00002	·00002	·00002	·00001	·00001	·00001	·00001
1·8	419·1	·00005	·00004	·00004	·00003	·00003	·00003	·00003	·00003	·00002	·00002
1·9	305·8	·00009	·00008	·00007	·00007	·00006	·00006	·00005	·00005	·00005	·00004
2·0	225·3	·00016	·00014	·00013	·00012	·00011	·00010	·00010	·00009	·00008	·00008
2·1	167·69	·00028	·00026	·00023	·00022	·00020	·00018	·00017	·00016	·00015	·00014
2·2	126·02	·00050	·00045	·00041	·00038	·00035	·00033	·00030	·00028	·00026	·00025
2·3	95·63	·00086	·00078	·00071	·00066	·00061	·00056	·00052	·00049	·00046	·00043
2·4	73·28	·00145	·00131	·00120	·00111	·00102	·00095	·00088	·00083	·00077	·00073
2·5	56·70	·00238	·00217	·00199	·00183	·00169	·00157	·00147	·00137	·00128	·00121
2·6	44·288	·00384	·00350	·00321	·00296	·00274	·00255	·00237	·00222	·00208	·00196
2·7	34·923	·00604	·00551	·00506	·00467	·00433	·00403	·00376	·00352	·00331	·00311
2·8	27·797	·00928	·00849	·00781	·00722	·00670	·00624	·00583	·00547	·00514	·00484
2·9	22·330	·01392	·01277	·01177	·01090	·01014	·00945	·00885	·00830	·00780	·00735
3·0	18·101	·02038	·01875	·01732	·01608	·01497	·01399	·01311	·01231	·01159	·01094
3·1	14·802	·02910	·02685	·02488	·02315	·02160	·02022	·01898	·01786	·01684	·01590
3·2	12·211	·04053	·03753	·03488	·03254	·03044	·02856	·02686	·02531	·02390	·02261
3·3	10·159	·05505	·05117	·04772	·04465	·04188	·03939	·03712	·03506	·03317	·03143
3·4	8·521	·07297	·06809	·06374	·05982	·05628	·05307	·05014	·04745	·04498	·04271
3·5	7·205	·09441	·08848	·08313	·07829	·07389	·06987	·06618	·06278	·05965	·05674
3·6	6·1394	·11934	·11232	·10595	·10014	·09481	·08991	·08540	·08122	·07734	·07373
3·7	5·2705	·14753	·13945	·13205	·12525	·11898	·11318	·10780	·10279	·09812	·09376
3·8	4·5571	·17854	·16947	·16111	·15336	·14616	·13946	·13321	·12736	·12187	·11672
3·9	3·9676	·21179	·20185	·19260	·18398	·17591	·16836	·16127	·15460	·14831	·14237
4·0	3·4770	·24656	·23589	·22589	·21649	·20766	·19933	·19146	·18402	·17698	·17029
4·1	3·0665	·28204	·27081	·26020	·25017	·24068	·23168	·22314	·21501	·20728	·19991
4·2	2·7206	·31742	·30578	·29473	·28421	·27420	·26465	·25554	·24684	·23852	·23055
4·3	2·4276	·35186	·33998	·32864	·31779	·30740	·29744	·28789	·27873	·26992	·26145
4·4	2·1780	·38457	·37261	·36112	·35008	·33945	·32922	·31937	·30986	·30069	·29184
4·5	1·9640	·41482	·40292	·39142	·38033	·36960	·35922	·34919	·33947	·33006	·32094
4·6	1·7797	·44198	·43025	·41887	·40784	·39713	·38674	·37664	·36683	·35729	·34802
4·7	1·6202	·46551	·45405	·44289	·43202	·42144	·41113	·40109	·39129	·38174	·37242
4·8	1·4814	·48496	·47385	·46299	·45239	·44202	·43190	·42199	·41231	·40284	·39357
4·9	1·3599	·50001	·48931	·47883	·46855	·45849	·44862	·43894	·42945	·42015	·41102
5·0	1·2533	·51044	·50020	·49014	·48026	·47054	·46100	·45162	·44240	·43333	·42441
5·1	1·1593	·51614	·50639	·49680	·48735	·47804	·46887	·45984	·45094	·44217	·43354
5·2	1·0759	·51709	·50787	·49876	·48978	·48091	·47216	·46353	·45500	·44658	·43827
5·3	1·0018	·51340	·50471	·49612	·48762.	·47923	·47092	·46271	·45459	·44657	·43863
5·4	0·9357	·50524	·49709	·48903	·48104	·47313	·46529	·45754	·44985	·44224	·43470
5·5	0·8764	·49289	·48529	·47775	·47028	·46286	·45551	·44822	·44099	·43382	·42671
5·6	0·8230	·47669	·46963	·46262	·45567	·44876	·44190	·43509	·42832	·42161	·41494
5·7	0·7749	·45706	·45054	·44406	·43761	·43120	·42484	·41851	·41222	·40597	·39975
5·8	0·7313	·43447	·42847	·42250	·41656	·41066	·40478	·39893	·39311	·38733	·38157
5·9	0·6917	·40942	·40393	·39846	·39302	·38761	·38221	·37684	·37149	·36617	·36087
6·0	0·6557	·38245	·37745	·37248	·36752	·36258	·35766	·35275	·34787	·34300	·33815
6·1	0·6227	·35410	·34958	·34508	·34059	·33611	·33165	·32720	·32276	·31834	·31393
6·2	0·5926	·32493	·32087	·31681	·31277	·30874	·30472	·30071	·29671	·29272	·28874
6·3	0·5649	·29546	·29183	·28821	·28460	·28099	·27739	·27380	·27022	·26664	·26308
6·4	0·5394	·26620	·26298	·25977	·25656	·25335	·25016	·24696	·24378	·24060	·23742
6·5	0·5158	·23760	·23476	·23193	·22910	·22628	·22346	·22064	·21783	·21502	·21222
6·6	0·4940	·21007	·20759	·20511	·20264	·20016	·19770	·19523	·19277	·19031	·18785
6·7	0·4739	·18394	·18179	·17964	·17749	·17535	·17320	·17106	·16892	·16679	·16465
6·8	0·4551	·15950	·15765	·15580	·15395	·15210	·15026	·14841	·14657	·14473	·14289
6·9	0·4376	·13694	·13536	·13378	·13220	·13063	·12905	·12748	·12591	·12433	·12276
7·0	0·4214	·11640	·11506	·11373	·11239	·11106	·10973	·10840	·10707	·10574	·10441
7·1	0·4062	·09794	·09682	·09570	·09458	·09347	·09235	·09123	·09012	·08900	·08789
7·2	0·3919	·08157	·08064	·07971	·07878	·07786	·07693	·07600	·07508	·07415	·07323
7·3	0·3786	·06724	·06647	·06571	·06495	·06419	·06342	·06266	·06190	·06114	·06038
7·4	0·3660	·05485	·05423	·05361	·05299	·05237	·05175	·05113	·05051	·04989	·04927
7·5	0·3543	·04428	·04378	·04328	·04278	·04228	·04178	·04128	·04078	·04028	·03978
7·6	0·3432	·03537	·03498	·03458	·03418	·03378	·03338	·03298	·03258	·03218	·03178
7·7	0·3327	·02796	·02765	·02733	·02702	·02670	·02639	·02607	·02576	·02544	·02513
7·8	0·3228	·02187	·02163	·02138	·02114	·02089	·02064	·02040	·02015	·01990	·01966
7·9	0·3134	·01693	·01674	·01655	·01636	·01617	·01598	·01579	·01560	·01541	·01521
8·0	0·3046	·01297	·01282	·01267	·01253	·01238	·01224	·01209	·01194	·01180	·01165
8·1	0·2962	·00982	·00971	·00960	·00949	·00938	·00927	·00916	·00905	·00894	·00883
8·2	0·2882	·00737	·00728	·00720	·00712	·00704	·00695	·00687	·00679	·00670	·00662
8·3	0·2806	·00547	·00540	·00534	·00528	·00522	·00516	·00510	·00504	·00497	·00491
8·4	0·2734	·00401	·00397	·00392	·00388	·00383	·00379	·00374	·00370	·00365	·00361
8·5	0·2665	·00291	·00288	·00285	·00282	·00278	·00275	·00272	·00269	·00265	·00262
8·6	0·2600	·00209	·00207	·00205	·00202	·00200	·00198	·00195	·00193	·00191	·00188
8·7	0·2538	·00149	·00147	·00145	·00144	·00142	·00140	·00139	·00137	·00135	·00134
8·8	0·2478	·00105	·00104	·00103	·00101	·00100	·00099	·00098	·00097	·00096	·00094
8·9	0·2421	·00073	·00072	·00071	·00071	·00070	·00069	·00068	·00067	·00067	·00066
9·0	0·2369	·00050	·00050	·00049	·00049	·00048	·00047	·00047	·00046	·00046	·00045

more difficult to perform than the ordinary fitting of a probit line. Its weakness is that it ignores any information on C contributed by test concentrations other than the controls. A low concentration of the poison may, on account of sampling variation, give a lower total mortality, p', than c, the mortality among the controls; this is an indication that c is an overestimate of the natural mortality, C. Similarly, values of p', at low concentrations, which greatly exceed c will indicate that c is an underestimate. A diagram of concentrations and total percentages killed will enable a rough curve to be sketched, from which an improved estimate of C may be taken. This may still be greater than some values of p', so that the application of Abbott's formula yields negative values for p; nevertheless the correct procedure is to calculate working probits by the usual rule, as, in spite of the fact that no meaning can be given to 'negative mortalities', this course permits all the observations to exercise their correct weight in the estimation of the probit regression line.

Maximum likelihood solution

When c is large and not determined with much higher precision than the mortalities under the poison, it may be desirable to carry out the complete maximum likelihood solution instead of the approximations discussed above. Using an estimate, C_0, of C, taken either as equal to c or as suggested by a sketch, a first approximation to the solution is obtained from the three simultaneous equations:

$$\delta C\left\{\frac{n_c}{C_0(1-C_0)}+\frac{1}{(1-C_0)^2}\,Snw\,\frac{Q^2}{Z^2}\right\}+a\,\frac{1}{1-C_0}\,Snw\,\frac{Q}{Z}+b\,\frac{1}{1-C_0}\,Snw(x-\bar{x})\,\frac{Q}{Z}=\frac{n_c(c-C_0)}{C_0(1-C_0)}+\frac{1}{1-C_0}\,Snwy\,\frac{Q}{Z},$$

$$\delta C\,\frac{1}{1-C_0}\,Snw\,\frac{Q}{Z}\qquad\qquad+a\,Snw\qquad\qquad=Snwy,$$

$$\delta C\,\frac{1}{1-C_0}\,Snw(x-\bar{x})\,\frac{Q}{Z}\qquad\qquad+bSnw(x-\bar{x})^2\qquad=Snw(y-\bar{y})\,(x-\bar{x}).$$

These equations are to be solved for δC, an adjustment to C_0 to give an improved estimate, and a, b, the constants of the probit regression line,

$$Y=a+b(x-\bar{x}).$$

To form the equations, p is first calculated from p' for each concentration, using C_0 in Abbott's formula, and the probits of p are used to give a provisional probit line. From this line, provisional probits, Y, are read to the nearest 0·1, and working probits, y, calculated by the usual rule. The weighting coefficients, w, are next found, using C_0 in the formula given earlier, and multiplied by n, the corresponding numbers of test organisms in the groups, to give the weights. The quantity Q/Z is independent of C_0, and is shown in Table 1; it is derived from Fisher & Yates' Table XI in which the maximum working probit, $Y+Q/Z$, is given. The symbol S denotes, as usual, summation over the various concentrations tested, x being the log concentration, and n_c is the number of test organisms used as controls.

Example. The computations may be illustrated on data from a trial of the toxicity of five different concentrations of pyrethrins to eggs of *Ephestia kühniella*. The concentrations were 0·05, 0·025, 0·01, 0·005, 0·0025 % of pyrethrins, the medium being 0·25 % w/v of sulphonated lorol in water, with 20 % v/v of acetone. Fifty eggs were left unsprayed, of which twenty-seven failed to hatch, and forty-nine were sprayed with the medium alone, twenty-eight failing to hatch. Since these two groups behaved so similarly, they have been combined to give a value of 0·5556 for c.

The first stages of the calculations are shown in Table 2. The first column, x, shows the log concentration, the concentrations having first been expressed in units of 0·001 % pyrethrins in order to avoid negative values here. Succeeding columns give the number of eggs exposed at each concentration (n), the number failing to hatch after treatment (r) and the proportion of non-hatching eggs (p'). Examination of the p' column, or of a rough sketch, indicates that the value of c given by the control batches is an underestimate of the mortality or inviability rate in the absence of pyrethrins. The sketch suggests 0·59 as a value in better agreement with the whole of the data, and this has been used in Abbott's formula to give the values of p in the next column. Empirical probits of p are read from Fisher & Yates's Table IX, and a provisional probit line drawn to give a series of provisional probits, Y.

The weighting coefficients are next calculated as described on p. 69, and multiplied by n to give the weights nw; tabulation of P is useful at this stage. The values of the working probit, y, are obtained from p and Fisher & Yates's Table XI in the usual way, and Q/Z is entered from Table 1. Product columns nwx, nwy, and nwQ/Z are then formed. These columns are multiplied in turn by x, y, and Q/Z to give the sums of squares and products shown in the lower part of Table 2. These are then adjusted to give sums of squares and products of deviations about means where necessary; thus $SnwxQ/Z$ is reduced by $\bar{x}SnwQ/Z$, and so on. The coefficients of the equations given earlier are obtained and the equations written as

$$\begin{aligned} 913\delta C+50{\cdot}5a-10{\cdot}93b &= 239{\cdot}66,\\ 50{\cdot}5\delta C+15{\cdot}70a &= 88{\cdot}481,\\ -10{\cdot}93\delta C+1{\cdot}1158b &= 4{\cdot}2868. \end{aligned}$$

TABLE 2. *Analysis of results of spraying eggs of* E. kühniella *with pyrethrins*

x	n	r	p'	$p(C_0=0{\cdot}59)$	Emp. probit	Provisional Y	Provisional P	nw	y	Q/Z	nwx	nwy	nwQ/Z
1·70	50	50	1·0000	1·0000	—	7·3	0·9893	1·54	7·68	0·379	2·6180	11·8272	0·5837
1·40	48	46	0·9583	0·8983	6·27	6·2	0·8849	6·77	6·27	0·593	9·4780	42·4479	4·0146
1·00	51	38	0·7451	0·3783	4·69	4·7	0·3821	6·59	4·69	1·620	6·5900	30·9071	10·6758
0·70	49	32	0·6531	0·1539	3·98	3·6	0·0808	0·787	4·09	6·139	0·5509	3·2188	4·8314
0·40	51	32	0·6275	0·0915	3·67	2·5	0·00621	0·0109	7·37	56·70	0·0044	0·0803	0·6180
$-\infty$	99	55	0·5556										
								15·70			19·2413	88·4813	20·7235

$\bar{x}=1{\cdot}225561$; $Snw\,Q/Z/(1-C_0)=50{\cdot}5451$; $n_c/C_0(1-C_0)=409{\cdot}26$; $n_c(c-C_0)/C_0(1-C_0)=-14{\cdot}0967$.

$Snwx^2$	$Snwxy$	$Snwy^2$	$Snwx\,Q/Z$	$Snwy\,Q/Z$	$Snw\,Q^2/Z^2$
24·6972	112·7260	515·6922	20·9177	0·41)104·0389	0·1681) 84·597
−23·5814	−108·4392	−498·6586	−25·3979	253·7534	503·3
			0·41)−4·4802	−14·0967	409·3
1·1158	4·2868	17·0336	−10·927	239·6567	912·6

These are best solved by the method outlined by Fisher (1941, § 29), as the inverse matrix of coefficients obtained provides the set of variances and covariances of the quantities δC, a and b. This matrix is

$$V=\begin{pmatrix} 0{\cdot}0015540 & -0{\cdot}004998 & 0{\cdot}01522 \\ -0{\cdot}004998 & 0{\cdot}07977 & -0{\cdot}04896 \\ 0{\cdot}01522 & -0{\cdot}04896 & 1{\cdot}0453 \end{pmatrix}$$

and the solutions of the equations are

$$\delta C=-0{\cdot}0046; \quad a=5{\cdot}650; \quad b=3{\cdot}797.$$

The new estimate of C is $(0{\cdot}5900-0{\cdot}0046)$, or 0·5854, and the probit regression line

$$\begin{aligned} Y&=5{\cdot}650+3{\cdot}797(x-1{\cdot}2256)\\ &=0{\cdot}996+3{\cdot}797x. \end{aligned}$$

Had this value of C differed substantially from the C_0 used in the calculations, or had the probit line given a series of probits very different from the provisional probits of Table 2, the next step would be to repeat the computations with these revised values, but, as will be seen shortly, the values obtained are sufficiently near the maximum likelihood estimates for practical purposes.

The value of Y at $x=1{\cdot}70$ is 7·451, corresponding to $P=0{\cdot}9929$, or, using the new value of C and Abbott's formula to calculate the expected value of the total proportion failing to hatch, $P'=0{\cdot}9971$. This gives an expectation of 49·9 inviable eggs. The full series of expected numbers of eggs hatching and failing to hatch is compared with the observed numbers in Table 3, and the agreement is seen to be good.

A test of significance of the departures of the observed numbers from the expected is given by squaring the difference between each 'expected' and the corresponding 'observed', and dividing by the observed to give a χ^2; since three parameters (C, a, b) have been estimated from the data, the degrees of freedom for χ^2 are three less than the number of concentrations, including the controls as a concentration. In making a χ^2 test the expected number in any class should not be very small, and consequently it is desirable here to group together the three highest concentrations to give observed numbers 15, 134, and expected 14·3, 134·7.* If this is done, only 1 degree of freedom remains, and $\chi^2=0{\cdot}93$; there is clearly no significant departure from expectation.

TABLE 3. *Observed and expected numbers of eggs*

Conc. of pyrethrins (%)	Total eggs	Observed Hatched	Observed Not hatched	Expected Hatched	Expected Not hatched
0·05	50	0	50	0·1	49·9
0·025	48	2	46	1·9	46·1
0·01	51	13	38	12·3	38·7
0·005	49	17	32	18·5	30·5
0·0025	51	19	32	21·0	30·0
Control	99	44	55	41·0	58·0

Since there is no evidence of heterogeneity, the elements of V are the variances and covariances of the parameters C, a, b; had χ^2 been significant, a factor of χ^2 divided by its degrees of freedom would have had to be introduced. The variance of C is thus 0·001554, and thus $C=0{\cdot}5854\pm0{\cdot}0394$, whence it is apparent that the adjustment, $\delta C=-0{\cdot}0046$, is small in comparison with the standard error and there is little advantage to be gained by further approxima-

* In the simpler case of probit analysis in which no allowance is made for mortality in the controls, χ^2 computed in this way from observed and expected numbers is identical with χ^2 derived from the sum of squares of deviations of y, as described in the Introduction to Fisher & Yates's Tables. The necessity of special treatment when either the number of survivors or the number killed at any concentration has a small expectation is present in this case also, though on account of the method of computation used for χ^2 this point has generally been ignored. The problem of making a satisfactory test of heterogeneity or departure from expectation in this situation deserves careful consideration, particularly as very many toxicity tests give an almost complete kill at the highest concentrations and the expected numbers of survivors are frequently less than 2.

tion to the maximum likelihood estimate. Similarly, the variance of b is 1·045, and $b = 3·797 \pm 1·022$, showing satisfactory agreement with the value taken for the provisional probits ($b = 3·7$).

As a further example of the use of the elements of V, the estimate of the median lethal dose may be considered. When C is large the two possible interpretations of the phrase 'median lethal dose' must be carefully distinguished, the first being the dose which will kill half of those test organisms which would otherwise have survived, and the second the dose which will ensure that only half of all the test organisms exposed will survive. The former seems the more useful meaning, especially as in some cases (as here) over half the test organisms fail to survive even in the absence of poison. The LD 50 is then given by

$$x - \bar{x} = (5 - a)/b \text{ or } x = 1·055 \pm 0·094,$$

the variance of this being

$$V(x) = \frac{1}{b^2}\{V(a) + 2(x - \bar{x})\,\mathrm{Cov.}\,(a, b) + (x - \bar{x})^2 V(b)\}$$

$$= \frac{1}{(3·797)^2}\{0·0798 + 2 \times 0·171 \times 0·0490 + (0·171)^2 \times 1·045\}$$

$$= 0·00882.$$

The covariance of a and b occurs as the third entry of the second column in the matrix V. The value of x corresponds to a concentration of $0·0114 \pm 0·0025\,\%$.

When two or more poisons are tested in a single experiment, and it is desired to compare their toxicities, the maximum likelihood equations given at the beginning of this section must be further elaborated in order to estimate the parameters for each poison. The analysis, though not difficult, is laborious; the experimenter who wishes to avoid its complexities will be well advised to use relatively large numbers of test organisms in the control batches if a heavy mortality is expected in these. When test organisms suitable for the experiment are plentiful, this should entail very little additional labour, but when they are scarce the choice of the most satisfactory proportions to be allotted to poisons and controls deserves careful consideration.

While preparing this paper I discovered that Prof. R. A. Fisher had reached similar results some years ago, but had not published them. I should like to express my thanks to him for help in discussing the problem, and also to Dr F. Tattersfield and Dr C. Potter for suggestions and for the use of their data on *E. kühniella*.

REFERENCES

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *J. econ. Ent.* **18**, 265–7.

Bliss, C. I. (1935). The calculation of the dosage-mortality curve. *Ann. appl. Biol.* **22**, 134–67.

—— (1939). The toxicity of poisons applied jointly. *Ann. appl. Biol.* **26**, 585–615.

Fisher, R. A. (1941). *Statistical Methods for Research Workers*, 8th ed. Edinburgh: Oliver and Boyd.

Fisher, R. A. & Yates, F. (1943). *Statistical Tables for Biological, Agricultural and Medical Research*, 2nd ed. Edinburgh: Oliver and Boyd.

Tattersfield, F. & Morris, H. M. (1924). An apparatus for testing the toxic values of contact insecticides under controlled conditions. *Bull. ent. Res.* **14**, 223–33.

(*Received* 31 *May* 1943)

Proceedings of the Association of Applied Biologists

Ordinary Meeting of the Association held on Friday, 1 October 1943, at 11 a.m. at the Imperial College of Science and Technology; the President, Prof. W. Brown, F.R.S., in the Chair.

Symposium on control of insects attacking stored foodstuffs

The following papers were read:

The organization and work of the Pest Infestation Laboratory of the Department of Scientific and Industrial Research. By G. V. B. Herford.

Factors influencing the course of an insect infestation in bulk wheat. By T. A. Oxley and R. W. Howe.

Behaviour of Tyroglyphid mite populations in stored grain and flour. By M. E. Solomon.

The role of the chemist in research on fumigation. By W. Burns Brown.

Control of the granary weevil with finely ground mineral dusts. By E. A. Parkin.

The organization and work of the Pest Infestation Laboratory of the Department of Scientific and Industrial Research

By G. V. B. Herford

Abstract

A brief historical account was given of the work on the control of the insect pests of stored produce in this country, leading up to the establishment by the Department of Scientific and Industrial Research of the Pest Infestation Laboratory.

For the period of the war, the Laboratory is housed in the Biological Field Station at Slough, the accommodation being generously provided by the Governing Body of the Imperial College of Science and Technology.

The principal aim of the Laboratory in war-time is to assist the Ministry of Food (Infestation Branch) in its efforts to control the insect pests of stored foodstuffs. After the war, although the greater part of the Laboratory's work will be still concerned with the pests of stored produce, it will not necessarily be confined to this particular field. Some of the work to be described is carried out in collaboration with other laboratories, e.g. the Cereals Research Station at St Albans and the Veterinary Research Laboratory at Weybridge.

The staff of the Laboratory, numbering thirty-five, are divided into three sections dealing with different aspects of the work, but remaining sufficiently elastic to allow of full and informal collaboration.

The sections are as follows:

Biology: Mr T. A. Oxley, in charge.

Insecticides: Dr E. A. Parkin, in charge.

Chemistry: Mr W. Burns Brown, in charge.

Biology Section. Deals with the life history and ecology of insects and mites and their effect on foodstuffs. In the case of grain this has necessitated a study of the physics and physiology of grain in bulk.

Special investigations include:

1. Estimation of infestation of a sample of grain by measuring the carbon dioxide production of the insects.
2. The heating of grain by insects and mites.
3. Improved methods of sampling grain, particularly at considerable depths, as in silo bins.
4. Adaptation of electrical method of measuring water content in a machine for separating the wetter portions from a stream of grain.

Insecticide Section. Deals with the effect of control measures on the insects, e.g. toxicity work on sprays, fumigants and insecticidal mineral dusts.

Special investigations include:

1. Biological evaluation of sprays.
2. Work on fly sprays (special war-time problem).
3. Study of the causes of variation in the resistance of insects to various control measures, especially to fumigants.
4. Study of the factors affecting the durability of toxic films of warehouse sprays.

Chemistry Section. Deals with fumigants in relation to the material to be fumigated and with their practical application in the field.

The work of the section is considered in some detail in a separate paper by Mr Burns Brown.

Factors influencing the course of an insect infestation in bulk wheat

By T. A. Oxley and R. W. Howe

(With 2 Text-figures)

The environment provided by bulk wheat in the British climate is far from ideal for the development of populations of the common grain insects. Some dominating characteristics of this environment are:

1. Air temperatures are normally well below the optima for development of most grain insects. Temperatures low enough to cause death are frequent but temperatures above the optima are rare.

2. Grain has a low thermal conductivity. Measurements made in this laboratory and reported more fully elsewhere (Oxley, 1944) have shown that that of wheat varies between 0·00036 and 0·00040 c.g.s. units.

3. Grain bulks are large. There are few other substances (and no other foodstuffs) which are commonly stored in such large homogeneous masses. As a result of this, and the low thermal conductivity, heat can only be lost with difficulty from the centre of wheat bulks and even seasonal variations in temperature do not reach the centre of some bulks.

4. The humidity of the intergranular air is determined by the equilibrium vapour pressure of the grain. In bulks of English wheat the relative humidity may be as high as 80–85%, but in imported wheats (which constitute the greater part of all bulks stored for long periods) the relative humidity is usually around 60%. This is low for most insects and is probably a restrictive factor for their development.

When an insect population develops in a bulk of wheat it modifies the environment in three chief ways:

1. Heat is produced.

2. Water is redistributed within the bulk and more water is produced by oxidation of hydrogen in the food (metabolic water).

3. Composition of the intergranular air is modified by production of carbon dioxide and consumption of oxygen.

It is important to assess the importance of these effects before describing the course of development of an insect infestation.

Heat production by an infestation

The rate of heat production by grain insects has not been measured, but it is possible to make rough estimates of the probable heat production based on the known rates of carbon-dioxide output. The latter is conveniently expressed as the 'carbon-dioxide figure' of the infested grain. The carbon-dioxide figure is the concentration, in per cent by volume, of carbon dioxide produced in 24 hr. at 25° C. in the intergranular air of a sample of grain which completely fills a sealed container. A full account of the determination and significance of the carbon-dioxide figure is given elsewhere (Howe & Oxley, 1944), but as a guide it may be stated here that a figure of 1% represents a rather mild infestation and 5–10% a heavy infestation. Infestations equivalent to several hundred per cent and even up to 1000% carbon-dioxide figure may occur for short periods in small quantities of grain and are common in ordinary insect cultures. Such high figures are determined by dilution of infested with uninfested grain or by the use of periods shorter than 24 hr.

The probable limits of heat production by such infestations may be estimated from the known heats of combustion of the three chief ultimate substrates of respiration. These are expressed below in terms of the weight of carbon dioxide produced instead of in the more usual terms of the weight of substrate oxidized.

Carbohydrate	2·6	g. calories	per mg.	CO_2 produced.
Protein	3·1	,,	,,	,,
Fat	3·4	,,	,,	,,

On this basis, the heat production lies between $1{\cdot}78 \times 10^{-7}$ and $2{\cdot}33 \times 10^{-7}$ calories per sec. per c.c. of infested grain, when the infestation is equivalent to a carbon-dioxide figure of 1%.

The temperatures reached may be calculated, assuming an evenly distributed infestation, using Awbery's formulae (Awbery, 1927), of which the appropriate form is:

$$t_1 - t_2 = \frac{Qr^2}{6K} \text{ at equilibrium;}$$

where r = radius of a sphere of grain in centimetres,
Q = rate of heat production of the grain in gram-calories per sec. per c.c.,
K = thermal conductivity of grain in c.g.s. units,
$t_1 - t_2$ = temperatures in degrees centigrade at the centre and periphery respectively of the sphere of grain.

Calculations based on these assumptions show that an infestation equivalent to 1% carbon-dioxide figure will not produce a significant temperature rise unless a very large bulk, e.g. 6 m. cube, is infested throughout to this extent. An infestation equivalent to a carbon-dioxide figure as high as 10–20%, however, will produce a considerable temperature rise in a bulk of grain only a metre cube. Temperature rises of a few degrees are frequently observed in insectary cultures, and these are only possible because of the exceedingly high density of population which develops in these cultures. 'Spontaneous' heating of grain due solely to the development of insect populations is a well-known phenomenon, and *Calandra* spp. appear to be the insects

most effective in causing it. Other insects have, however, been found to be responsible in a number of instances.

A rise of temperature causes an increase in the rate of heat production, so that a self-accelerating process is initiated, but this can only proceed to a limiting maximum temperature which must be set by the temperature tolerance of the insects concerned. In fact the highest temperature which we have observed in numerous studies of 'hot spots' caused by insects is very constant and lies between 38–42° C. This temperature is surprisingly high, since the maximum temperature for development of *Calandra* is about 35° C. and the maximum for oviposition is about 32° C. Thus *Calandra* is evidently able to heat grain to temperatures well above its own maximum temperature for development.

It seems probable that the maximum temperature for insect heating of grain is determined by the lethal temperature of the insects concerned. This presumably, therefore, lies between 38–42° C., and the actual lethal temperature may depend, among other conditions, on the rate at which it is approached.

Water relations of an infestation

The vapour pressure of water in grain rises with temperature. Hence, when a temperature gradient is set up in a bulk of grain a gradient of water-vapour pressure is also set up. Movements of air by diffusion or convection therefore result in movement of water down any temperature gradients, so that hot grain becomes gradually drier and surrounding cool grain becomes damper.

Owing to the very slow diffusion of air through grain, transfer of water is unimportant except when temperature gradients are very steep, as they are when a hot patch occurs close under the surface of a bulk or near to metal girders which have a high thermal conductivity. In such cases the rise in water content of thin layers of surface grain may be very great indeed, resulting in caking, sprouting, and finally mould development and rotting. This phenomenon is responsible for most of the spectacular results of 'heating' of grain, and the obvious excess of moisture at the surface in such cases leads many people to assume that dampness of the grain was the cause rather than the consequence of insect infestation.

The phenomenon of dampness surrounding patches of grain heated by insect activity has been said by some authors to be due to the production of metabolic water. A simple calculation, however, shows that the role of metabolic water is insignificant, since the maximum amount of water which could be produced by oxidation of any food is one molecule per molecule of carbon dioxide. On this basis, the water content of grain will be raised by approximately 4×10^{-4} % per day by an infestation equivalent to 1 % CO_2 figure.

Thus, even a relatively heavy infestation producing a carbon-dioxide figure of 7 % will take about a year to raise the water content of the grain by 1 % if no water is lost.

Infestations dense enough to raise the water content of grain appreciably by metabolic water can only exist in very small patches a few centimetres in extent, since lethal temperatures will be reached very quickly in larger bulks. Such densities are reached in cultures of *Calandra* and other insects, and the water content of these does in fact rise several per cent in a single generation.

Gas exchange of an infestation

We have frequently determined the carbon-dioxide concentrations developed in the intergranular air of infested grain. Normally, concentrations considerably less than 1 % by volume have been found, and we have no evidence that such concentrations have any effect on insect metabolism or reproduction.

Evidence derived from determinations of carbon-dioxide concentrations at various depths shows that this gas is normally removed and oxygen replenished chiefly by convection. Convection, however, can only operate when there are distinct hot and cool regions through which the upward and downward currents respectively can pass. This condition exists when heating is sharply localized into hot patches, but when the whole of a considerable bulk is heating little convection occurs and carbon-dioxide concentrations of 4–5 % or higher may easily occur even in a relatively shallow bulk stored on a floor. Widespread heating, however, is a late stage of a process which normally begins in patches and it very seldom happens that grain bulks are allowed to reach this condition.

Carbon dioxide sometimes also accumulates in conditions of semi-airtight storage, such as iron or concrete tanks or silos, even when these are open at the top. Containers of this sort are usually comparatively narrow and there is little room for convection currents in two directions, so that gas exchange occurs chiefly by the much slower mechanism of diffusion. A deep silo, however, will not hold carbon dioxide at a concentration above a few per cent unless the lower openings are unusually airtight. Owing to its density an atmosphere containing carbon dioxide will pour out of a deep silo bin through even a very small leak and a majority of silos are not made airtight.

When conditions permit accumulation of carbon dioxide (and a corresponding depletion of oxygen) this will become a controlling factor in an infestation. An example of this has been fully described by Howe (1943) in an open-topped iron bin only 4 ft. cube.

The progress of an infestation

We may now consider the probable progress of an infestation within a bulk of grain.

Suppose that a small number of adult insects are present within a bulk of otherwise clean grain and at least 50 cm. from the surface of the bulk. If the temperature of the grain is above the minimum for oviposition (i.e. about 12° C. for *Calandra* spp.) eggs will be laid. If the temperature is below the minimum for oviposition, but not too low, adults of most insects are able to survive for several months without ageing appreciably and will be able to lay eggs when the temperature becomes suitable.

If the temperature of the grain is below 20° C. development of most grain pests will be very slow indeed and *Calandra* spp. may take 120 days to complete their life cycle at the relative humidities usual in dry wheats, i.e. around 60%. In grain damper than usual, periods down to 60 days may be expected.

Temperatures higher than 20° C. are not normally maintained in this country for more than a few months, and hence it is quite possible that the first generation will be interrupted by the onset of winter before it is complete. This is probably the most serious hazard which besets the precarious early stages of an infestation.

In winter, temperatures near 0° C. will usually be reached by January or February throughout all but the largest bulks of grain. Such temperatures are fatal to all stages of *Calandra oryzae* and to all stages but the adults of *C. granaria*, and even these will usually die if exposed for 2 months or more to temperatures near zero. Probably larval stages of most grain insects die under winter conditions in this country.

If an infestation in its first generation is not completely eliminated by winter temperatures the sole survivors will be old adults. These, however, will probably be capable of oviposition and, since their eggs will be laid in early summer, the chance of survival beyond the first generation will be very high.

If the infestation is near the centre of a very large bulk of grain, minimum winter temperatures may never be reached and some larvae or pupae may survive. Survival of the first generation will, of course, be aided if there is any artificial heating.

The chief hazards of establishment of an infestation are over when most of the first batch of larvae reach their fourth instar (*Calandra*), since they produce considerable heat at this stage and the temperature rise which this causes hastens their own development to maturity and accelerates the development of the younger ones. The amount of temperature rise will be closely related to the density of the population.

Since at low temperatures a rising temperature increases metabolism, and hence heat production, a self-accelerating process is initiated as soon as a population begins to develop. As a result, any slight differences in the original density or date of egg laying, or in local conditions of humidity or temperature, become enormously exaggerated and the typical patchy development of infestation in 'pockets' is produced.

It is probable that much of the patchiness of insect population development is due to this cause. A similar result can also be caused by the mixing of grain of various densities of infestation in the course of ordinary grain-handling processes. It is impossible at present to assess the relative importance of these two causes.

Once temperatures above 18–20° C. have been established in this way, deep in a bulk, the infestation can develop in spite of winter temperatures outside. So long as the temperatures are below the optima for development and metabolism, multiplication is most rapid at the thermal centre of a developing infestation. Hence temperature rise is very rapid at the centre and the optimum is soon passed. Since larvae of *Calandra* and *Rhizopertha* live within the grains, they are unable to leave an unfavourable region; the temperature therefore continues to rise to levels which are highly unfavourable to the insects and eventually becomes lethal. This appears also to be true, at least in part, when the infesting insects are free living and are presumably free to migrate, perhaps because the temperature rise is too rapid for most of the insects to make their escape.

Although the insects are killed and presumably produce no more heat, the temperature does not normally fall, since the same process of heat production leading to sterilization is continuing in an ever enlarging sphere around the central core so that there is no cooler grain available to which heat can be lost. Exceptions occur when the hot region is close to the edge of a bulk and the infestation does not progress equally in all three dimensions away from the centre. Such an exception is illustrated in Fig. 2, where a cool path existed in a horizontal direction towards a nearby wall.

A developing infestation thus produces highly unfavourable conditions, becoming fatal, at the centre of the infested region. It is surrounded, however, by a falling temperature gradient in which optimum conditions for development are present, not only of temperature but also probably of humidity, since translocation of water tends to dry the central core and to raise the water content of the cooler surrounding grain. In the zone of optimum conditions development is very rapid, so that rapid extension of the infested area is encouraged. This fact, coupled with the tendency for all free-living forms to migrate from excessively hot, dry conditions to cooler and damper regions, is probably a major factor in promoting the spread of infestations once they are well established.

We have observed hot patches in various stages of this development. The number of insects, both adults and young stages, present in grain samples from regions hotter than 38° C. has always been very

small. Sometimes sterilization appears to be almost complete.

A typical example of such data from a mass of heating grain in which the latest stages of the above process had been reached is given in Table 1. Very large numbers of living insects were present on the surface and down to a small depth. The temperature of the grain rose from air temperature at the surface to 38·9° C. at 100 cm. depth, and this temperature persisted to the greatest depth investigated. *Latheticus*, *Oryzaephilus* and *Laemophloeus* were present in small numbers, but *Calandra oryzae* and *Rhizopertha dominica*, both of which are forms whose larvae live inside the grains, were the chief infesting insects. Samples were drawn from each of the depths indicated and the carbon-dioxide figure of these was determined in the laboratory. Dr O. W. Richards (Imperial College of Science and Technology) kindly dissected 200 grains from each of the samples and the larvae which he found are given in columns 3 and 4 of Table 1.

TABLE 1. *Larvae and adults of* Rhizopertha dominica *and* Calandra oryzae *in samples from a hot, heavily infested, bulk of wheat*

Depth (cm.)	Grains holed (%)	No. larvae found by dissection in 200 grains		Living adult insects per sample			CO_2 figure (%)
		C. oryzae	*R. dominica*	*C. oryzae*	*R. dominica*	Psocids	
0	11·5	16	3	Many	Many	—	11·5
15	8·5	11	0	3	1	—	1·4
25	6·0	6	1	0	7	—	6·2
50	7·5	8	3	0	3	—	4·7
75	6·0	5	1	0	0	Present	0·5
100	5·5	10	3	0	0	,,	0·3
150	6·0	9	0	0	0	,,	0·4
200	5·0	2	2	0	0	,,	0·4
250	7·5	5	7	0	0	,,	0·4
300	5·5	6	1	0	0	,,	0·6
400	4·5	5	2	0	0	,,	0·5

Grains were soaked in alcohol before dissection and it was not possible to be certain which larvae had been alive, but the sharp fall in the carbon-dioxide figures of samples from 75 cm. and below shows that all insects except psocids in these deeper samples were dead. The presence of larvae in all samples, however, and the presence of holed grains from which adult insects had emerged, show that in fact there had previously been a living infestation at all depths investigated.

The living psocids in the deeper samples are sufficient to explain the small carbon-dioxide figures actually obtained. The survival of psocids at such high temperatures confirms other observations which we have made on the distribution of these insects.

Other features of the progress of deep-seated infestations are illustrated in Figs. 1 and 2.

Fig. 1 represents a vertical section through part of a bulk of grain in which a high infestation of *Laemophloeus* sp. has developed. The solid lines are isotherms drawn at intervals of 5° C. The broken lines are extra isotherms drawn at 37·5 and 41° C. in order to make clear the shape of the heating area. The figures indicate the numbers of *Laemophloeus* adults and larvae which were obtained from 60 g. samples taken at the points indicated by the black dots.

The arrangement of the isothermal lines is strongly suggestive of a recent rapid rise of the hottest zone, and this is confirmed by the small numbers of insects found in samples taken from immediately below this zone. The steep temperature gradient over the hot zone was marked by an area of caked and slightly mouldy grain at the surface of the bulk. It is clear that the greatest numbers of insects were obtained from samples taken in the favourable regions just outside the hottest zone. The large insect population adjacent to the floor about 1·5 m. from the bulkhead is probably the result of some local favourable condition which is not revealed by the data obtained. Heating is not developed here because of the relatively high rate of heat loss through the floor.

Laemophloeus is more tolerant of high temperatures than *Calandra* and hence large numbers were able to survive near the surface in the hottest zone. This is probably partly because the full temperature had been only very recently developed at this point and the population revealed by the samples was probably migrating upwards since *Laemophloeus* is free living at all stages.

Fig. 2 shows the temperatures recorded at a particular point in an infested bulk of wheat on three occasions. The infestation originated at floor level and we were not able to observe it until conditions at the bottom of the bulk were near the end of the cycle of events described above. The slight convexity at the bottom of the 4 Mar. curve reflects the falling off in heat production above 35° C. The curve for 1 Apr. shows that about half the grain had reached the maximum temperature for infestation

by that date. The curve for 22 Apr. shows some cooling as a result of death of the insects between depths of 175 and 260 cm. This heat was lost horizontally to an adjacent wall; heat could not have been lost vertically to regions at higher temperatures and in fact cooling of this kind is only rarely observed.

The curves show clearly the rapid rise in temperature of the cooler upper layers of the grain, the infestation at each depth passing through the development cycle previously described. The existence of a rather definite maximum temperature is well illustrated.

The progress of an infestation on the surface

At the surface of a bulk of grain, temperature and humidity are controlled entirely by the atmosphere of the warehouse. In the winter, humidity conditions are likely to be much more favourable than

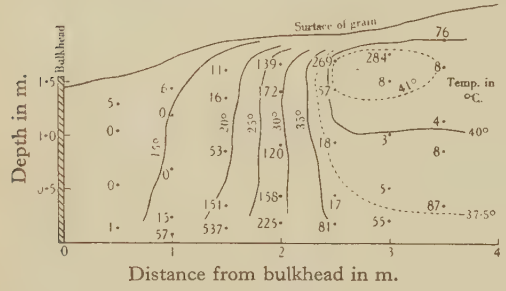


Fig. 1. Vertical section through a bulk of grain showing isotherms and numbers of *Laemophloeus* spp.

in the depth of the bulk, but the temperature is too low for the development of any of the more important grain insects. In summer the humidity drops in most situations until it is little, if any, more favourable than in the bulk, but the temperature reaches a level sufficient for the multiplication of insects much earlier in the summer than it does in the bulk. Hence, superficial infestations are able to start early in the summer at the surface of grain and may progress through several generations during one summer under favourable conditions.

The onset of winter will kill all stages of the infestation except adults, though in a cold winter many or all of these will die also. This will occur however great the multiplication has been during the summer, since the heat produced by the insects cannot accumulate to cause a rise in temperature. Hence, every winter only those insects which have migrated into the bulk early enough to establish their first generation at a considerable depth will survive. Thus the infestation will cease to be superficial each winter, though a new superficial infestation may be established each summer from any population which may have survived in the depth.

Apart from direct injury to grains, most of the damage which an infestation causes is a consequence of heating. A superficial infestation is thus relatively

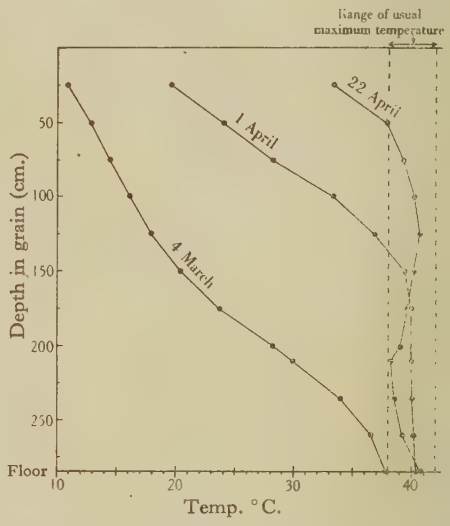


Fig. 2. Graph showing temperatures on 4 Mar., 1 Apr. and 22 Apr. 1943, at various depths in a bulk of wheat infested with insects. In this instance the infestation began at floor level.

unimportant except as a source of insects which can establish deep infestations. A surface infestation cannot by itself cause heating, caking, sprouting, mould development, or rotting of grain.

Unimportant superficial infestations of grain bulks are relatively common, but serious deep-seated infestations are rather rare. It is evident that a number of factors frequently operate to prevent the transition of an infestation from unimportant to epidemic numbers, and an analysis of some of them is the aim of part of our present work at the Pest Infestation Laboratory.

REFERENCES

AWBERY, J. H. (1927). The flow of heat in a body generating heat. *Phil. Mag.* **4**, 629.

HOWE, R. W. (1943). An investigation of the changes in a bin of stored wheat infested by insects. *Bull. ent. Res.* **34**, 145.

HOWE, R. W. & OXLEY, T. A. (1944). The use of carbon dioxide production as a measure of infestation of grain by insects. *Bull. ent. Res.* (in the Press).

OXLEY, T. A. (1944). The properties of grain in bulk. 3. The thermal conductivity of wheat, maize and oats. *J. Soc. Chem. Ind.* (in the Press).

Behaviour of Tyroglyphid mite populations in stored grain and flour

By M. E. Solomon

Abstract

Investigations of aspects of this problem were described, and discussed in relation to relevant published data.

(1) *Damage done.* Baking tests on artificially infested flour indicated a high degree of tolerance to *Tyroglyphus farinae* L.: e.g. after 25 days an infestation which had increased to 0·263 g. mites per 100 c.c. flour caused no perceptible deterioration in baking quality. The effects of older infestations have yet to be tested. Damage to grain is usually restricted to destruction of the embryo; this significantly reduces the nutritive value of the grain. Indirect damage includes contamination of the materials. Other workers have described intestinal, respiratory and skin disorders caused by mites.

(2) *Distribution.* Tyroglyphids are almost ubiquitous, and are readily spread by wind, birds, rodents or man. The *hypopial* stages are specially adapted for distribution by insects or for resistance to unfavourable conditions.

(3) *Effects of physical conditions on survival and multiplication.* Humidity plays the dominant role. Figures were given showing the correlation between the moisture content of grain and the occurrence and density of mite populations. The rate of multiplication increases strikingly as the humidity increases; the optimum temperature range is usually about 18–25° C. (One species has been reported to multiply 3-million-fold in 3 months under favourable conditions.) Tyroglyphids are very susceptible to desiccation, and cannot live in grain or flour if the moisture content is below about 12% (≡R.H. 55–60%). They are very resistant to low temperatures, but are killed by suitable exposures to temperatures of 40° C. and over.

(4) *Effects of physico-chemical conditions on movement and distribution of mites.* Results of an experimental study of the behaviour of tyroglyphids were shown to be of value in explaining their movements in stored materials. Preference for high humidity is the chief factor.

(5) *Interactions between tyroglyphid populations and predators.* Data from granary investigations were given, showing that populations of *Tyroglyphus farinae* L. increased rapidly during the winter and spring, but dwindled or disappeared during summer and autumn, due to the predatory activities of the mite *Cheyletus eruditus* Schr. The seasonal cycle was due to the differential effects of physical conditions on the two species; this was confirmed in laboratory experiments. This example of the intimate relationship between a predator/prey cycle and the physical environment is interesting in relation to recent population theory, in which the general tendency has been to separate the biological and physical factors.

The role of the chemist in research on fumigation

By W. Burns Brown

In the study of the properties and methods of application of insecticides there is work for the chemist, the physicist and the engineer as well as for the biologist.

A study of the literature dealing with the special field of fumigation of stored products suggests that frequently the biologist and the chemist have pursued their researches independently of one another. The biologist has naturally shown much more interest in the behaviour of his biological material than in the chemical and physical behaviour of the fumigant, and he has tended to employ biological methods of investigation where chemical or physical methods would frequently have been more appropriate. The scope of his deductions has been limited by his incomplete understanding of the behaviour of the fumigant in purely chemical or physical terms. It is here that the chemist can make a most valuable contribution, but it is essential that the biologist and the chemist should work in the closest possible collaboration.

As far as research in the Slough laboratories is concerned the credit for initiating and encouraging such a collaboration is undoubtedly due to Prof. Munro, who, in 1929, arranged for the appointment of Dr Page as research chemist to work with the entomologists in his department of the Imperial College of Science on practical control measures to be used against stored products pests. Under Page's direction a strong chemical section was built up and maintained throughout those pre-war years. Largely as a result of his influence the work on fumigation at Slough has taken on certain characteristics which mark it out as differing from that carried out else-

where. Following the inception of the Pest Infestation Laboratory of the Department of Scientific and Industrial Research, absorbing as it did a nucleus of staff from the Imperial College Biological Field Station, it was natural that the research on fumigants should continue without interruption along the lines which had already proved so productive of useful data.

The most important of the characteristics just mentioned is an almost complete division of the work on fumigants into two parts. A predominantly biological section studies the behaviour of the fumigant upon insects and determines its toxicity under carefully controlled conditions, in the absence, as far as possible, of any sorptive material. Meanwhile, the chemical section studies the behaviour of the fumigant alone, either in small-scale laboratory tests or in commercial-scale fumigations in large chambers, warehouses, barges and ships. Where possible in the large-scale fumigations, observations of the effect upon the natural insect population are made, but the use of tubes or cages of insects, as commonly employed by other workers to test the efficacy of the treatment, is avoided.

By a combination of the data obtained by the two sections many of the diverse problems put to the Laboratory have been solved satisfactorily. This division of the work is only made possible by an insistence on the determination of concentrations of fumigant in the air at all stages of the work. In the great bulk of the work on fumigation carried out elsewhere the concentrations of fumigant in the air are not determined, and the characteristic feature of such work is an endeavour to relate directly the dosage of fumigant applied under very diverse conditions with the mortality observed in tubes or cages of 'test insects', or with the effect upon the product fumigated.

Referring first to the determination of toxicities, a large part of the published data has been obtained with apparatus of simple design having a gas reservoir of small dimensions. This makes accurate dosage difficult and does not permit of the taking of samples of the air for the determination of concentrations. Moreover, losses of fumigant upon rubber connexions, grease on stopcocks and even upon the insects may become important when the volume of gas used is small, and the concentration to which the insects are exposed may be only a fraction of the nominal concentration calculated from the dose applied and the volume of the apparatus. Some workers have carried out toxicity tests in small chambers constructed of wood or other absorptive materials without making any check of the actual concentrations present. In fact very few workers have considered it necessary to determine actual concentrations in toxicity work. The need for the determination of these concentrations becomes more urgent when the period of exposure is extended. Much of the published data relates only to exposures of 5 hr. or less. When toxicities are determined for exposures of 18, 24 or 48 hr., the normal periods of treatment given in commercial work in this country, the dose to be applied is correspondingly reduced and the losses due to sorption by the apparatus may be more serious. Some workers present data showing the so-called 'toxicity' of a fumigant to insects in the presence of a quantity of some sorptive material such as grain or flour. These are not true toxicity data. They are presumably intended as a direct guide to the doses to be applied in practical fumigations, but I have rarely found them of any assistance in this direction. A large proportion of the published toxicity data has been obtained at 25° C. or higher temperatures. There is very little data for temperatures below 20° C. Yet, in this country, the majority of commercial fumigations are carried out at temperatures below 20° C. and on occasions the temperature may be below 10° C. Thus for the full investigation of practical fumigations on the lines followed at Slough there is need of reliable toxicity data at the lower ranges of temperature and for the longer periods of exposure. These data must be for 100% mortality.

The apparatus for the determination of toxicities at present in use at Slough was designed by Page and Gough. It has a gas reservoir of about 130 l. capacity, the gas being continuously circulated through four pairs of insect chambers which can be cut out of the system in pairs at the times required. The whole apparatus is enclosed in a cabinet maintained at constant temperature, refrigeration being available for the lower ranges of temperature. Concentrations of fumigant are normally determined at the start and end of each experiment.

While experiments at higher temperatures, e.g. 25° C., and shorter exposures, e.g. 5 hr., may be justified on the grounds of economy of time and ease of working, for preliminary comparative work on different fumigants and insects or for studying the behaviour of the insects and the causes of variation of resistance, the experiments should eventually be extended to cover ranges of temperature and periods of exposure more nearly related to those applying in normal practical fumigations. The temperature factor is especially important.

The relation known commonly as Habers's rule for the action of poisons on mammals has been shown to apply as a first approximation to the action of fumigants on insects. This states that the toxic effect is proportional to the product of the concentration of fumigant multiplied by the time of exposure. Where the concentration is varying continuously, as in a practical fumigation, the concentration-time integral or the area under the concentration-time curve may be taken as a measure of the toxic effect obtained at the given point. For a given fumigant and insect the concentration-time product

giving 100% mortality does show some variation, however, over wide ranges of exposure time, so that in correlating laboratory-obtained toxicity data with the concentration-time products observed in a practical fumigation the periods of exposure in each case should be of the same order.

Determination of concentrations of fumigant in the air are made in the Slough laboratories by collecting a known volume of air in an evacuated vessel. This may contain an absorbing solution or be provided with means for adding it later. The fumigant in the solution is determined subsequently. Alternatively all or part of the air and fumigant collected in the vessel may be transferred to a gas-volumetric apparatus or passed through some additional decomposition and sorption system. The method of sampling has been considerably developed and has proved to be applicable to many different fumigants and in widely differing conditions. It has many advantages over the more commonly used method of aspirating a known volume of air through bubblers containing absorbing solutions. In particular, it facilitates the collection of a large number of samples within a short period of time. In general, when determining concentrations of fumigant in the intergranular spaces of packed goods the gas sample collected should be as small as possible. The larger the sample the greater the error introduced by drawing air from surrounding areas.

Before undertaking the investigation of some commercial-scale fumigation process it is customary to carry out tests in the laboratory on a single package or on a few packages of the material requiring treatment. These tests are carried out in steel chambers in which loss of fumigant by leakage or by sorption on the walls can usually be neglected. In such a test the penetration of fumigant is studied by determining concentrations at different points in the package and in the free space outside the package.

Normally, one finds that after the application of the fumigant the concentration in the free space falls steeply as sorption in the material takes place. The hindrance to penetration caused by the wrapping materials can be determined by comparing the concentrations found at the inner surface of the wrapper with those found at corresponding times in the free space. Apart from such hindrance, penetration to the centre of the material may be very slow. Thus, in a test of this kind on a 140 lb. bag of wheat flour using ethylene oxide as fumigant, 24 hr. elapsed after the introduction of the vaporized fumigant before the concentration at the centre of the bag approximated to that in the free space. Concentration-time products can be obtained for each point sampled and can be compared with the product shown by the toxicity data to be necessary under the conditions of the fumigation for complete mortality of any insects present. The period of fumigation necessary to obtain this lethal concentration-time product at any particular point in the package can at once be seen. In a series of tests in which the dose of fumigant or the method of application is varied, the effect of these factors upon the period of fumigation necessary for effective treatment can be accurately determined.

By continuing to determine concentrations at points in the package after the nominal end of the fumigation, i.e. during the period of airing, an accurate measure of the so-called 'post-fumigation' effect can be obtained. The concentration at the centre of the bulk, particularly if the material be finely divided, may fall off slowly over a period of several days, as the fumigant is desorbed from the material. It is in those products which offer the greatest resistance to penetration by the fumigant, and which are therefore most difficult to treat successfully, that this post-fumigation effect will be most marked.

In a series of tests under comparable conditions on a given product with different fumigants, a comparison of the concentration-time curves for the free space gives an indication of the relative rate and amount of the sorption of each fumigant by the product. The curves for a given point inside a bulk of the material indicate the rate of penetration of each fumigant, and the areas under these curves give a measure of the efficiency of penetration. The two sets of curves are closely linked, since in most cases sorption is the chief factor governing the rate of penetration.

In laboratory tests of this type or in commercial-scale trials the amount of information to be gained by the use of test insects, in the absence of any determinations of gas concentrations, is, even under the best conditions, comparatively limited. Yet test insects are very widely used for comparison of different techniques of fumigation. In any one experiment the mortality of insects obtained can give an indication of the effectiveness of the treatment only over the total period of the fumigation without giving any clear picture of the behaviour of the fumigant throughout the period. If 100% mortality is obtained it is impossible to say to what extent the minimum lethal treatment has been exceeded. If less than 100% mortality is obtained, additional toxicity data must be available if one is to judge how far the treatment falls short of that required for complete mortality. The chief objection to the use of test insects in this way, however, is the very considerable variations in resistance shown by different batches of the same species of insect. This alone renders insect resistance unsuitable as a measure of the effectiveness of a particular treatment. Some information has already been obtained on the causes of the variation shown, particularly in regard to the treatment of the insects immediately before exposure, but, undoubtedly, much further work

requires to be done on the more fundamental causes of variation. In the measurements of toxicity under carefully controlled laboratory conditions some indication of the extent of this variation in a particular insect can be obtained and can be taken into account in arriving at a figure for the concentration-time product which it is desirable to attain in a practical fumigation.

The great importance of sorption in practical fumigations, so amply demonstrated by data showing the variation of concentration with time, has not been fully realized by many workers. As a result considerable confusion exists in the literature between the dosage applied and the actual concentrations obtained. This confusion is made the more serious by reason of the method most commonly adopted for the expression of dosage, namely, a weight of fumigant for a given gross volume of the space treated. Thus the dosages of various fumigants recommended for the treatment of different products in chambers and warehouses are commonly expressed in pounds per 1000 cu. ft. of space. These recommendations take no account of the proportion of the space occupied by the product. In the majority of cases, that is, when the fumigant is highly sorbed by the product, the dose based on the volume of the chamber must be very much greater when the space is well filled with goods, than that necessary to give equal concentrations when a small proportion of the space is so occupied. It would be better in many such cases to base the dose upon the weight of goods to be treated, but the only system of dosage which is universally applicable is one combining both these systems. Thus a certain weight of fumigant should be allowed per ton of product and an additional weight of fumigant should be allowed per 1000 cu. ft. of the space unoccupied by goods. This system has been successfully applied in this country for the fumigation of dried fruits, wheat, flour, nuts and other products with ethylene oxide.

A very large field of work is open to the chemist in the study of the sorption and retention of fumigants by different products. This work occupies the largest proportion of the time of the Chemistry Section of the Pest Infestation Laboratory and I cannot attempt to discuss it in this brief survey. The work includes the devising of analytical methods suitable for determining the amount of fumigant sorbed, the use of these methods in the study of the amount and rate of sorption under various conditions, the mechanism of this sorption (which may be both physical and chemical), and the rate of desorption under various conditions.

Allied with this work on sorption is the investigation of damage to the product. This work falls under various heads according to the nature and properties of the product. With some food products there may be a danger of taint; with cereals or cereal products the milling and baking quality may be affected; in foodstuffs residues of fumigant may remain which are harmful to humans or to farm stock. Most of these investigations involve collaborative work with other research institutions.

Unfortunately the value of much of the published data upon the retention of fumigants and the damage to products is greatly reduced because of the inadequate description of the fumigation treatment given to the product. This description is frequently limited to a statement of the dose of fumigant applied (expressed, for instance, as pounds per 1000 cu. ft. of space) and the period of fumigation. As has been shown, the gas concentrations actually obtained in such a test, and hence the effects to be expected, will vary widely according to the amount of product in the space treated. In all such tests the weight of product and the volume of the space should be recorded as well as the dose applied, and a series of gas concentrations in the free space should be determined.

Control of the granary weevil with finely ground mineral dusts

By E. A. Parkin

(*Abridged account*)

Introduction

Nearly three years ago, the Insecticide Section of the Pest Infestation Laboratory started an investigation into the possibility of utilizing finely ground mineral dusts for control of the granary weevil, *Calandra granaria* L. Valuable preliminary work on the subject had been carried out by the Chemical Research Laboratory, Teddington. The problem was one of considerable war-time importance in relation to the storage in this country and other parts of the Empire of large quantities of grain over abnormally long intervals of time. I wish to say that I speak also for two other members of my staff who have been fully engaged upon this problem, namely, Dr N. L. Spoor, who has been responsible for the physico-chemical side of the investigation, particularly for particle-size determination and segregation, and Mr A. M. Cunnington, who has carried much of the

weight of setting up experiments to measure insecticidal efficiency, including their periodic examinations for estimation of insect mortality.

The outstanding advantage of dust treatment is that, after application, the dust remains in contact with the grain until removed during the cleaning processes preliminary to milling and should continue to exert its insecticidal action over a long period. Moreover, there is no taint and no change in the milling quality of the grain, nor is germination affected. Dusts can be applied by unskilled labour or simple mechanical means. The disadvantages of dust treatment are that the free-running property of grain is somewhat reduced, assessment of the quality of grain is hampered and the presence of mineral dust intensifies the dust cloud which usually rises when grain is handled.

During the past 15 years several papers have been published upon the efficacy of various dusts and their mode of action, notably by Zacher & Kunike (1930), Germar (1936), and Chiu (1939).* These accounts deal only with laboratory work. No account has yet been published of a large-scale trial of a dust, but some authors have concluded from laboratory experiments or small-scale trials that, in practice, mineral dusts are not likely to be so good or so convenient as might be expected (Claus, 1939; Ratcliff *et al.* 1940; Cotton *et al.* 1942).

I wish now to summarize the work on dusts undertaken at the Pest Infestation Laboratory. The laboratory work was done as rapidly as possible in order to be able to suggest dusts for early practical trial and was therefore not always as detailed or coherent as we would have wished, but was planned to meet the needs of the moment.

With few exceptions, assessment of the insecticidal power of different dusts under various conditions has been made by determining the death-rate of 100 adult *Calandra granaria* confined on 100 g. of Manitoba wheat which was previously thoroughly mixed with 1% by weight of the dust under test. The insects were bred under controlled conditions and were 3–4 weeks old when used: insects, grain and dusts were all conditioned to the desired temperature and humidity—generally 25° C. and 70% R.H.—before the start of the experiment. Tests were replicated several times in each experiment. Time-mortality curves were generally plotted and found to be sigmoid in shape, as expected, but often considerably skewed.

Preliminary sorting tests

With the help of the Geological Survey and Museum, and Raw Materials Department, Ministry of Supply, samples were obtained of twenty-two materials available in this country during war-time and including such diverse substances as natural minerals, flue dusts from power stations and blast furnaces, power-station clinker, etc. Substances not already finely powdered were ground by pestle and mortar to pass a 300-mesh sieve, but the particle-size distributions of the various materials were not known. From the results of these tests, twelve materials were considered worthy of further study.

Later sorting tests

More detailed consideration of the problems of supply led to the omission from further trial of six of the twelve materials and the inclusion of an alternative six. In order to ensure more comparable particle-size distributions the substances were specially ground in a commercially operated 'Micronizer' (Lissman, 1938). Analysis of the particle-size distributions by a modified Andreasen sedimentation technique revealed that the harder materials were not so finely ground as the softer.

Mortality counts were made at intervals upon weevils confined on dusted grain, and from this and other ancillary tests the most promising materials appeared to be felspar, silica and dolomite; silica preparations were later ruled out from use on a practical scale because of the inherent silicosis hazard.

Whilst this work was proceeding to select materials worthy of extended trial, other experiments were being undertaken to gain information upon the biological and physico-chemical factors influencing the insecticidal efficiency of mineral dusts.

Biological factors influencing mortality

(1) *Experimental technique.* It was early suspected that the disturbance of the insects during the counting of mortality increased the rate of action of the dusts; this was proved experimentally. The interval between examinations should therefore be kept constant in any experiment or series of experiments to compare the relative speeds of kill of a number of different dusts or different particle-size fractions of the same dust. Alternatively, single jars from a series should be examined once only at selected intervals. It was also concluded that dusts are likely to act more slowly in practice when applied to grain which is not disturbed over long periods.

(2) *Stage of weevils.* The immature stages of *C. granaria* are passed within the grains and have been shown to be unaffected by the external coating of dust.

(3) *Age of weevils.* The resistance of adult *C. granaria* in weekly age groups from 0–1 to 5–6 weeks after emergence was found to rise to a maximum in the 3–4 weeks' group, followed by a slight fall in the next 2 weeks.

(4) *Sex of weevils.* There was no significant difference in resistance of males and females.

(5) *Specificity.* Although *C. granaria* was used

* See also Briscoe, H. V. A. (1943). Some new properties of inorganic dusts. *J. roy. Soc. Arts*, 91 (4650), 593–607.

throughout as the test insect, *Tribolium confusum*, *Rhizopertha dominica* and *Oryzaephilus surinamensis* were included in one test with four mineral dusts of widely different chemical and physical properties, namely flint, felspar, limonite and anhydrite. Broken grain was employed because *T. confusum* and *O. surinamensis* are unable to feed on whole grain. Flint proved to be the most effective dust against all four species of insects and *O. surinamensis* was more susceptible to the action of all four dusts than the other three species. The remaining data indicated that the order of insecticidal efficiency of a number of dusts changes with the species of insect used in the test but more evidence is clearly desirable on this aspect of the problem.

Tyroglyphus farinae, a mite commonly found in grain and cereal products, has been found to be very susceptible to the action of finely ground mineral dusts. When the mite occurs in abundance, however, the relative humidity of the atmosphere is often too high for really effective control by application of these substances.

Physico-chemical factors influencing mortality

(1) *Chemical composition.* It has not been possible to obtain any clear evidence of a relation between chemical composition and insecticidal action, chiefly because of the difficulty of differentiating the effect of physical differences between the various materials. Some are almost completely ineffective even when extremely finely ground.

(2) *Concentration.* Although the general conclusion can be drawn that increase in concentration increases the speed of kill, the relation between these two factors may be very different for different dusts. In dusts of a wide particle-size range it is probable that the really effective fraction is that below about 10 μ in diameter. Results achieved in experimental work may therefore depend more upon the concentration of this fraction than upon the concentration of the dust as a whole when applied to grain.

Apart from concentration, the effectiveness of a given material is dependent also upon its particle-size distribution. Thus, for a given degree of control it should be possible to choose between a certain concentration of fine dust and a higher concentration of coarser dust. The chief factors governing the choice will then be the concentration of dust necessary and its cost of production at the required particle size.

(3) *Particle-size distribution.* A considerable proportion of the laboratory work has been devoted to a study of the relation between particle-size distribution and insecticidal efficiency. This has led to the amassing of a large body of data which has proved difficult to interpret. Some experiments were carried out with a series of six sedimented fractions of quartz dust of very narrow particle-size range and approximate mean particle diameters of 0·5, 1·8, 3·2, 5·5, 10·0 and 15 μ. When applied to grain at 1% by weight, the 1·8 μ material was the most effective against *C. granaria*. Similarly the loss in weight and the mortality of starving weevils dusted with these fractions were greatest with the 1·8 μ quartz.

Down to a limiting degree of subdivision effectiveness increases with fineness, and in very effective ground dusts with normal particle-size distributions the proportion by weight of particles of diameter larger than 30 μ is negligible. If a long period of grinding is required for the production of a fine dust, insecticidal efficiency may under some circumstances be reduced. In these instances other factors, e.g. particle shape or change of the surface structure, have probably been introduced.*

(4) *Particle shape.* There is reason to suspect that particle shape (including, perhaps, surface structure) may influence the insecticidal efficiency of dusts but, because of the difficulty of obtaining samples of ultra-finely divided materials of the same particle-size range, yet of different particle shape, it has not been possible to undertake confirmatory experiments. The general effectiveness of very hard materials (see below) may be related to their tendency to yield on grinding angular, microcrystalline particles, but it must be borne in mind that some amorphous materials, e.g. flint and precipitated colloidal silica, are also very effective.

(5) *Hardness.* Using sedimented fractions of the same particle-size range (0–5 μ), effectiveness has been shown to increase with increasing hardness. The cost of grinding, however, also rises with increasing hardness.

(6) *Relative humidity.* The rate of death of weevils on carefully conditioned grain treated with 1% by weight of a micronized felspar decreased rapidly with increasing relative humidity. At 100% R.H. the dusts become completely ineffective.

(7) *Temperature.* At a constant relative humidity of 70%, a rise in temperature within the range investigated (10–30° C.) accelerated the action of the dust.

(8) *Retention of activity.* It was shown that the efficiency of dusts was not impaired by application to grain and storage at constant temperature and relative humidity for periods up to 6 months.

Application of dusts to grain

When it is desired to protect uninfested grain stored in bulk, it may be sufficient to deposit the grain on a floor well covered with dust and then shovel a thick layer of dust over the surface of the pile. If insect infestation is suspected, intimate mixing of grain and dust must be undertaken. Small quantities of grain, e.g. seed stock, can be treated by shovel mixing or in a rotating drum mixer as used for animal feeding stuffs. When grain-handling

* See also Briscoe (1943), *loc. cit.*

machinery is installed, large bulks can be treated by introducing the dust at a measured rate into the grain stream just before its deposition on the storage floor. The precise method of feeding in the dust will depend on the design of the whole plant but may be by hopper devices, worm conveyor, etc.

Removal of dusts from grain

An early small-scale experiment carried out on our behalf by the Cereals Research Station, St Albans, showed that there was likely to be no difficulty in removing dusts from wheat during the ordinary cleaning processes through which it passes before milling. This was later confirmed by an experimental run in a flour mill.

The organic detritus removed during the cleaning of grain is utilized in animal feeding stuffs and the mineral dust from treated grain would be collected with this organic fraction. By the time the dusted fraction has been mixed with other materials in the preparation of the final mash, etc., the concentration of mineral dust will have been reduced to a level at which no real risk would be involved, at least with dusts like anhydrite, felspar and dolomite.

Mode of action of dusts

The precise mode of action of these mineral dusts is still unknown and it is probable that their gross effect represents a combination of several factors.

Weevils confined on grain treated with an effective dust soon become coated with the dust which accumulates particularly in the folds of the intersegmental membranes, in the joints of the legs and in the mouth-parts. The insects become very slow in movement and are unable to progress normally. Several workers, including ourselves, have found that dusted insects lose water more rapidly than untreated controls.

The pros and cons of various theories to account for the mode of action of mineral dusts may now be considered.

(1) *Mechanical action.* The adhesion of dusts to the body surface and especially their accumulation in the intersegmental membranes and joints of the limbs cause a considerable hindrance to movement and probably also to copulation and oviposition. Whilst this mechanical action of dusts must help to restrict the spread of infestation, it cannot be regarded as a primary cause of death.

(2) *Starvation.* Complete blockage of the mouth-parts will lead to death from starvation, but this factor can be of little or no importance, since in the absence of food dusted weevils succumb more rapidly than undusted.

(3) *Desiccation.* Several workers have observed that dusted weevils lose weight at a greater rate than undusted. This weight loss is caused by a reduction in the water content of the insects. The presence of grain reduces the speed of action of dusts, probably because the intake of food enables the insects to compensate to a small extent for excessive water loss. Since the water must evaporate from the insect into the atmosphere, the importance of relative humidity, or more properly saturation deficiency, becomes readily understandable.

The question now arises, what influence has an effective mineral dust on an insect to induce this abnormal water loss? Zacher & Kunike (1930) suggest that enormous increase in the surface of the insect by the adherent dust will give a greater area for evaporation. This view is not supported by the result of one of our experiments with quartz-sedimented fractions of narrow particle-size range. The weight of dust which persistently adhered to 100 weevils was measured for the different fractions and the increase in surface area calculated approximately. The increase was greatest for the finest fraction, which was shown to be less insecticidal than the next coarser fraction. Increase in surface area may aid water loss, but is certainly not the primary cause of desiccation.

Germar (1936) concluded that effective dusts become tightly pressed in the intersegmental grooves, and water is removed by capillary effects from the intersegmental membranes and passed to the atmosphere. There is no evidence, however, to show that liquid water can be drawn through the insect cuticle by non-hygroscopic dusts. If the intersegmental membrane were penetrated by sharp particles, haemolymph should escape. Dust has been recovered from the intersegmental folds of larvae of *Tenebrio molitor* and tested, without positive result, for the presence of proteins.

Apart from a temporary initial stimulation, dusts decrease the activity of beetles, and Germar (1936) rightly discounts the idea that the higher rate of water loss indicates a greater rate of metabolism. More recently, measurements of the oxygen consumption and carbon-dioxide output of dusted and undusted insects have shown no increases due to dusting.

The most likely explanation of the insecticidal action of mineral dusts appears to be interruption of the continuity of the water-retaining lipoid layer of the cuticle by adsorption on to the dust particles. This lipoid layer forms the outer surface of the cuticle and prevents undue loss of water from the insect. If the layer is wholly or partly removed and its replacement prevented by continued adsorption on to the dust particles, water will be able to evaporate more easily through the body wall. Nearly all the experimental data can be accounted for on this basis, and the few items presenting difficulty are those most in need of confirmation. Until much more is known of the physico-chemistry of the insect cuticle and of dust particles, however, it will probably be impossible to explain the variations in the order of susceptibility of a series of insects to a given group of dusts.

REFERENCES

CHIU, S. F. (1939). Toxicity studies of the so-called 'inert' materials with the rice weevil and the granary weevil. *J. econ. Ent.* **32**, 810.

CLAUS, A. (1939). Über weitere Prüfungen des Präparates Naaki als Kornkäferbekämpfungsmittel im Jahre 1938. *Z. angew. Ent.* **26**, 165.

COTTON, R. T., WAGNER, G. B. & WINBURN, T. F. (1942). Insecticidal value of certain dusts for the protection of stored grain. *J. Kansas ent. Soc.* **15**, 1.

GERMAR, B. (1936). Versuche zur Bekämpfung des Kornkäfers mit Staubmitteln. *Z. angew. Ent.* **22**, 603.

LISSMAN, M. A. (1938). Jet pulverising now practical. *Chem. metall. Engng*, **45**, 238.

RATCLIFF, F. N., GAY, F. J. & FITZGERALD, J. S. (1940). Wheat weevils and their control: a summary of existing information. *J. Coun. sci. industr. Res. Aust.* **13**, 229.

ZACHER, F. & KUNIKE, G. (1930). Untersuchungen über die insektizide Wirkung von Oxyden und Carbonaten. *Arb. Biol. Reichsanst.* **18**, 201.